

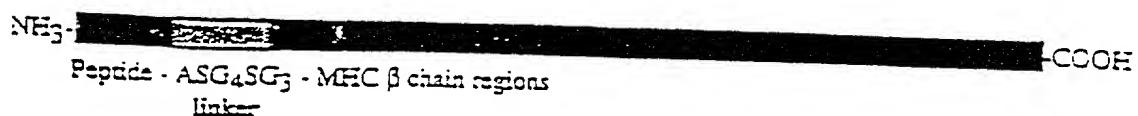
Figure 1A. Peptide-linker- $\beta$  chain construct

Figure 1B. Schematic view of peptide-linked MHC binding groove

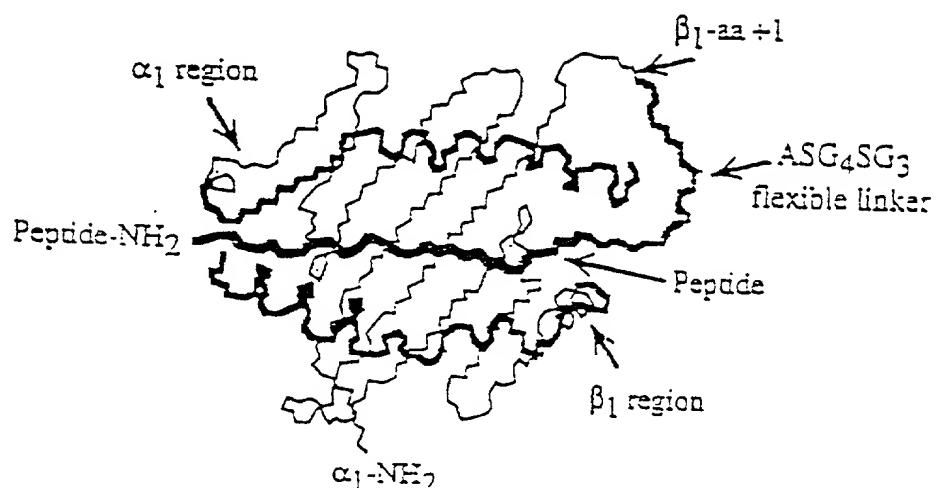
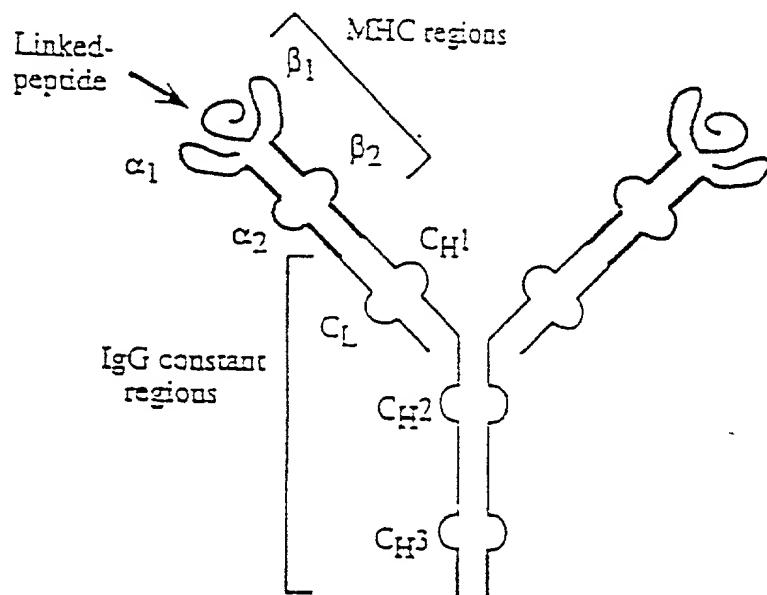


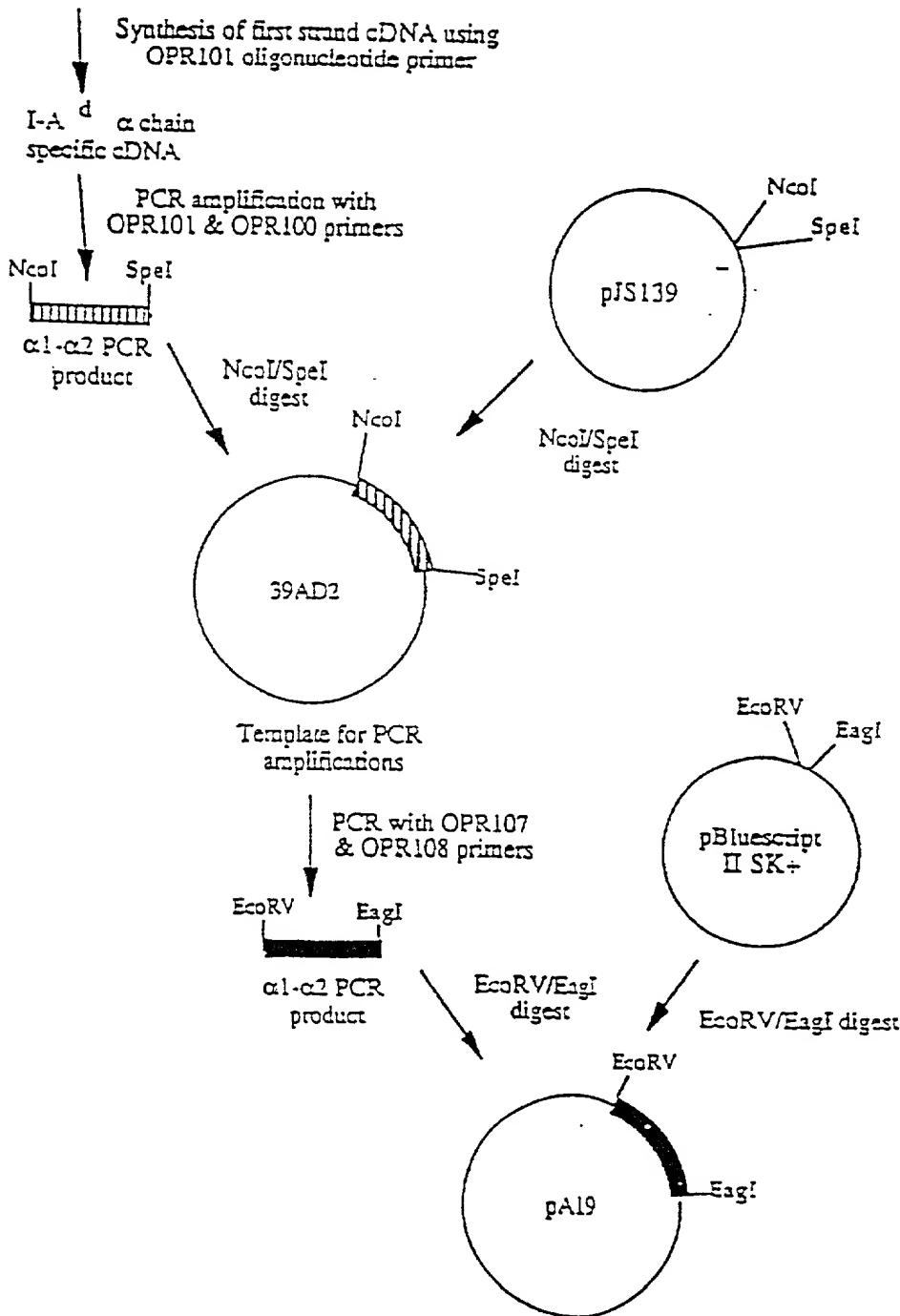
Figure 1C. Schematic view of soluble peptide-linked MHC-IgG C-region fusion protein



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Figure 2: I-A<sup>d</sup>  $\alpha$  chain cloning scheme

Total RNA isolated  
from A20 cells



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Figure 3: I-A<sup>d</sup>  $\beta$  chain cloning scheme

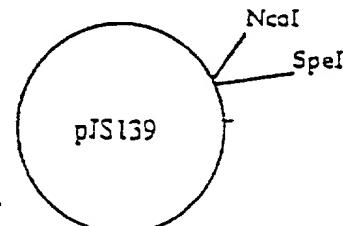
Total RNA isolated  
from A20 cells

↓  
Synthesis of first strand cDNA using  
oligo dT primer

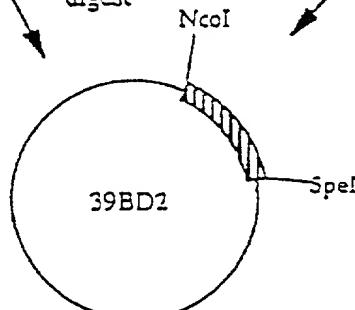
cDNA template

↓  
PCR amplification with  
OPR102 & OPR104 primers

NcoI      SpeI  
\_\_\_\_\_  
 $\beta$ 1- $\beta$ 2 PCR  
product



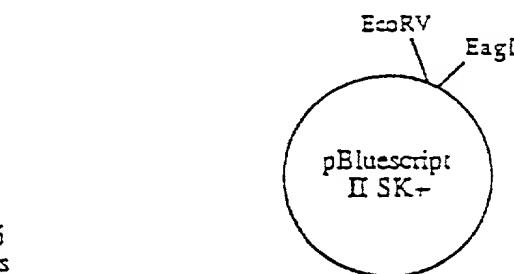
NcoI/SpeI  
digest



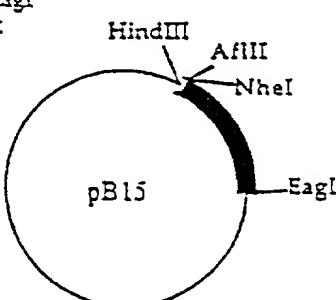
Template for PCR  
amplifications

↓  
PCR with OPR106  
& OPR112 primers

EcoRV      EagI  
\_\_\_\_\_  
NheI  
AflII  
Linker- $\beta$ 1- $\beta$ 2  
PCR product



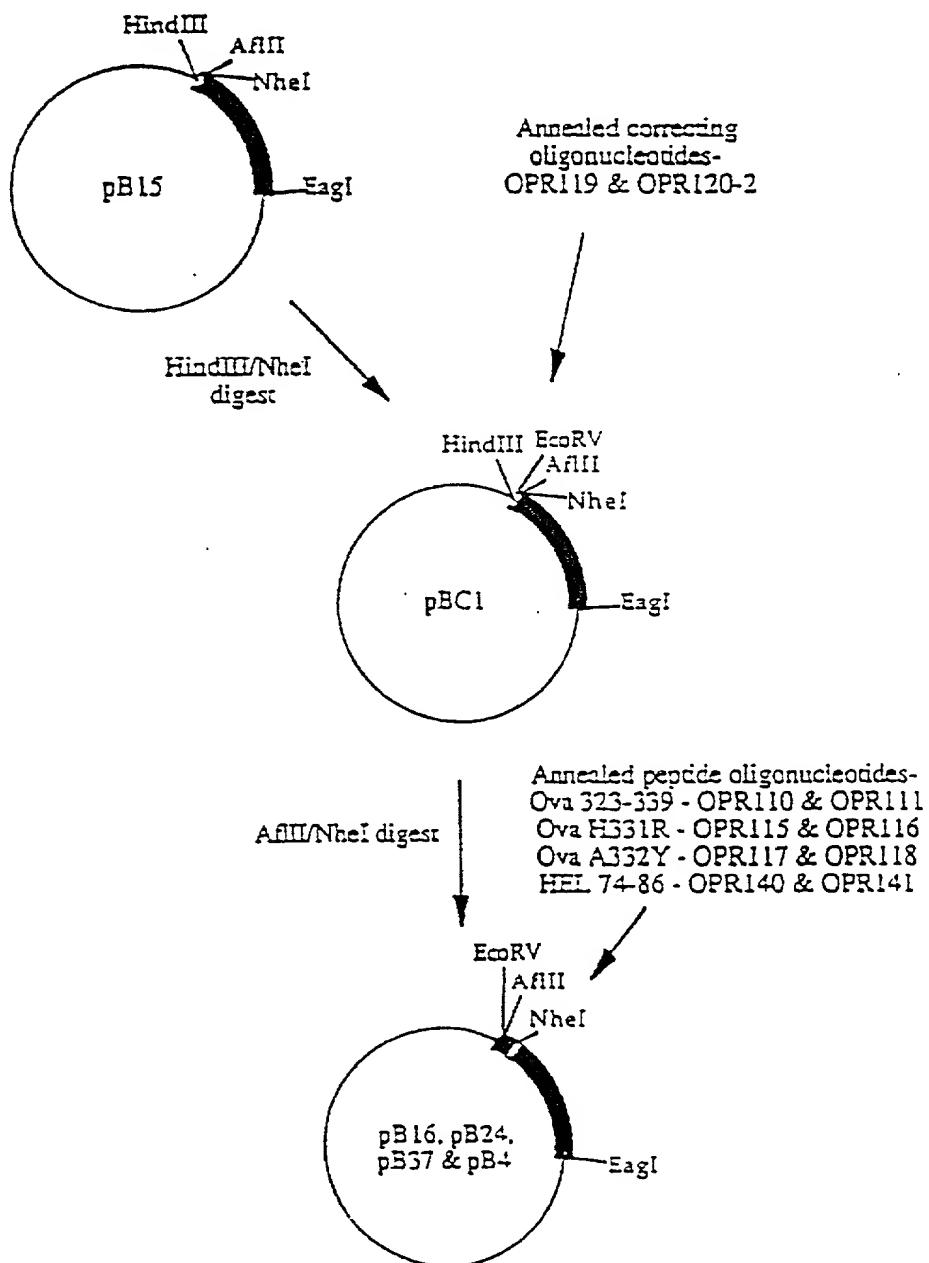
EcoRV/EagI digest



Mutation in linker region-  
no EcoRV site present

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Figure 3: cont.



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Figure 4: HLA-DR1  $\alpha$  chain cloning scheme

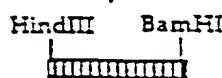
Total RNA isolated  
from BLCL K68 cells

↓  
Synthesis of first strand cDNA using  
oligo-dT primer

cDNA  
template

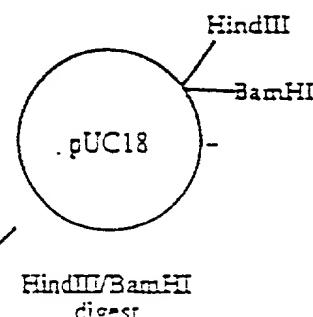
↓  
PCR amplification with  
DRI1A-B & DRI1A-F primers

HindIII      BamHI



$\alpha 1$ - $\alpha 2$ -hinge  
PCR product

HindIII/BamHI  
digest



HindIII  
digest

HindIII/BamHI  
digest

K68A3

BamHI

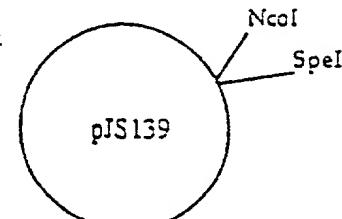
Template for PCR  
amplifications

↓  
PCR with AF-N &  
AB-S primers

NcoI      SpeI

$\alpha 1$ - $\alpha 2$ -hinge  
PCR product

NcoI  
digest



SpeI

↓  
NcoI/SpeI digest

NcoI

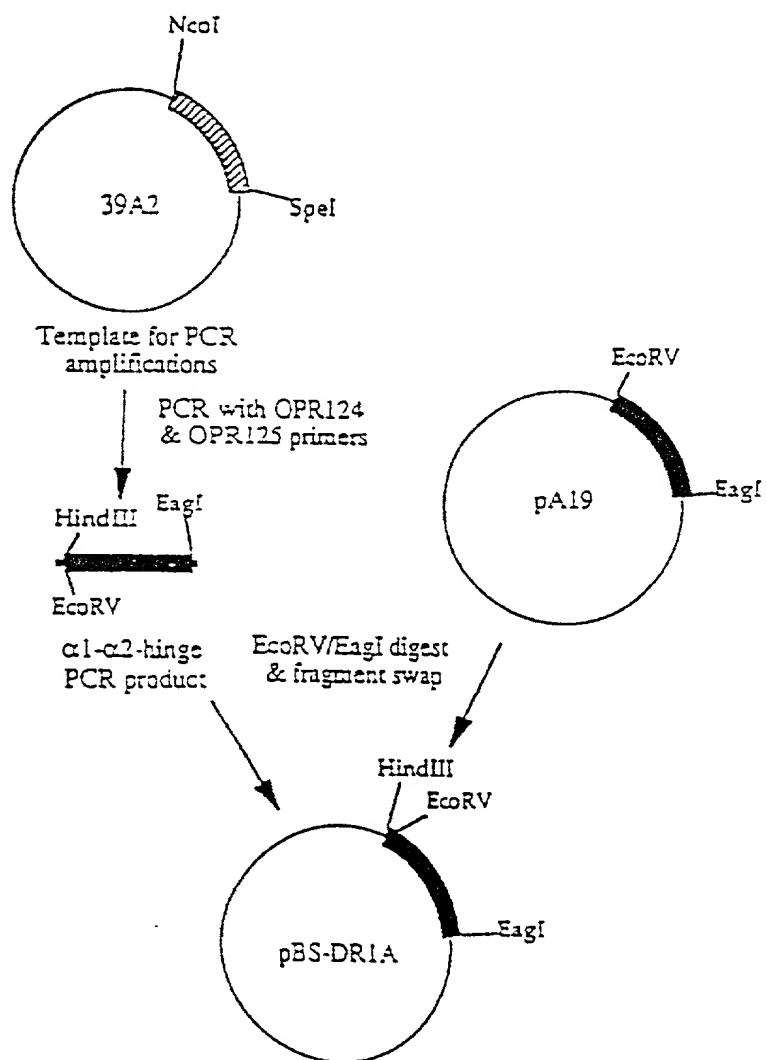
39A2

SpeI

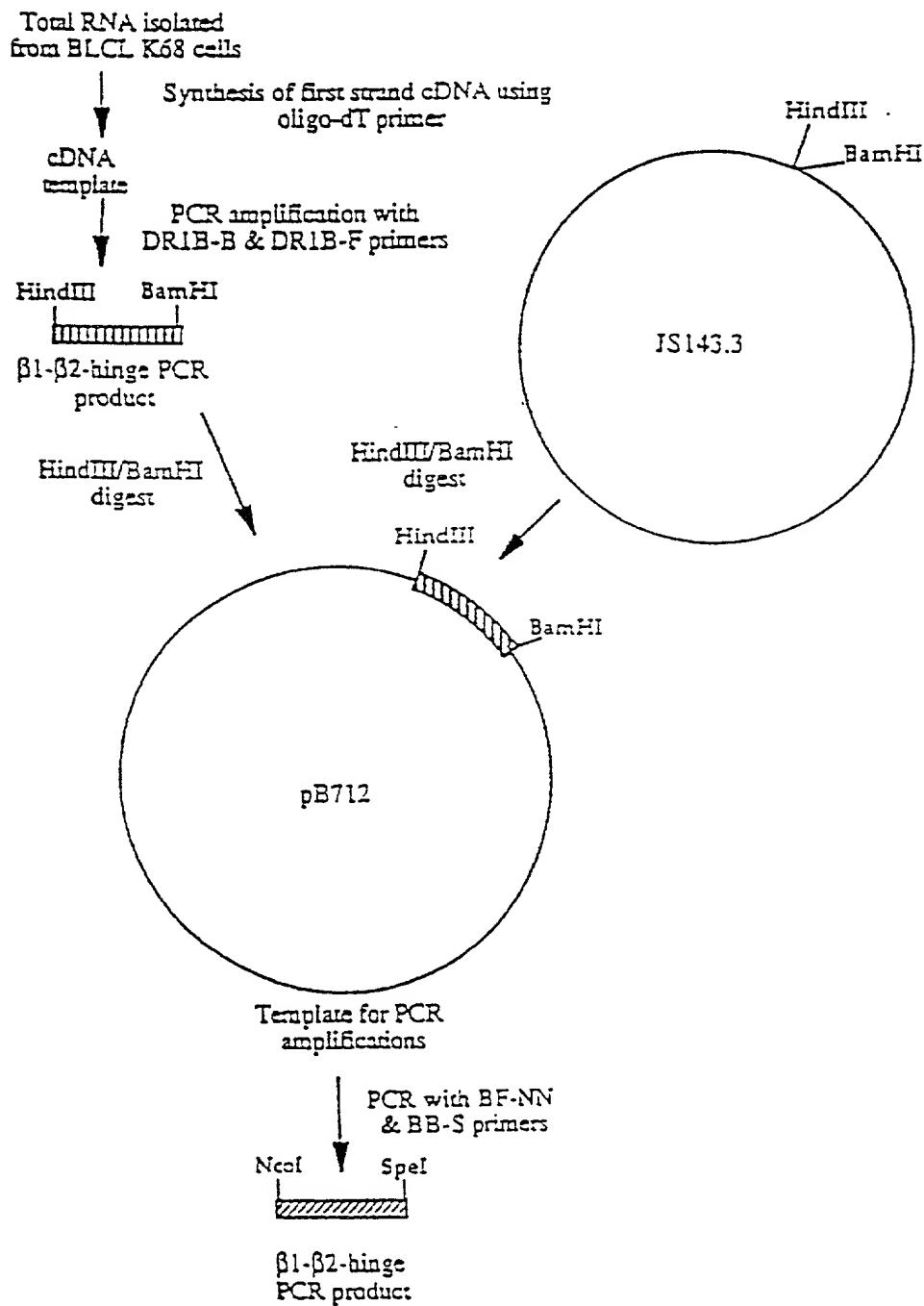
Template for PCR  
amplifications

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Figure 4: cont.

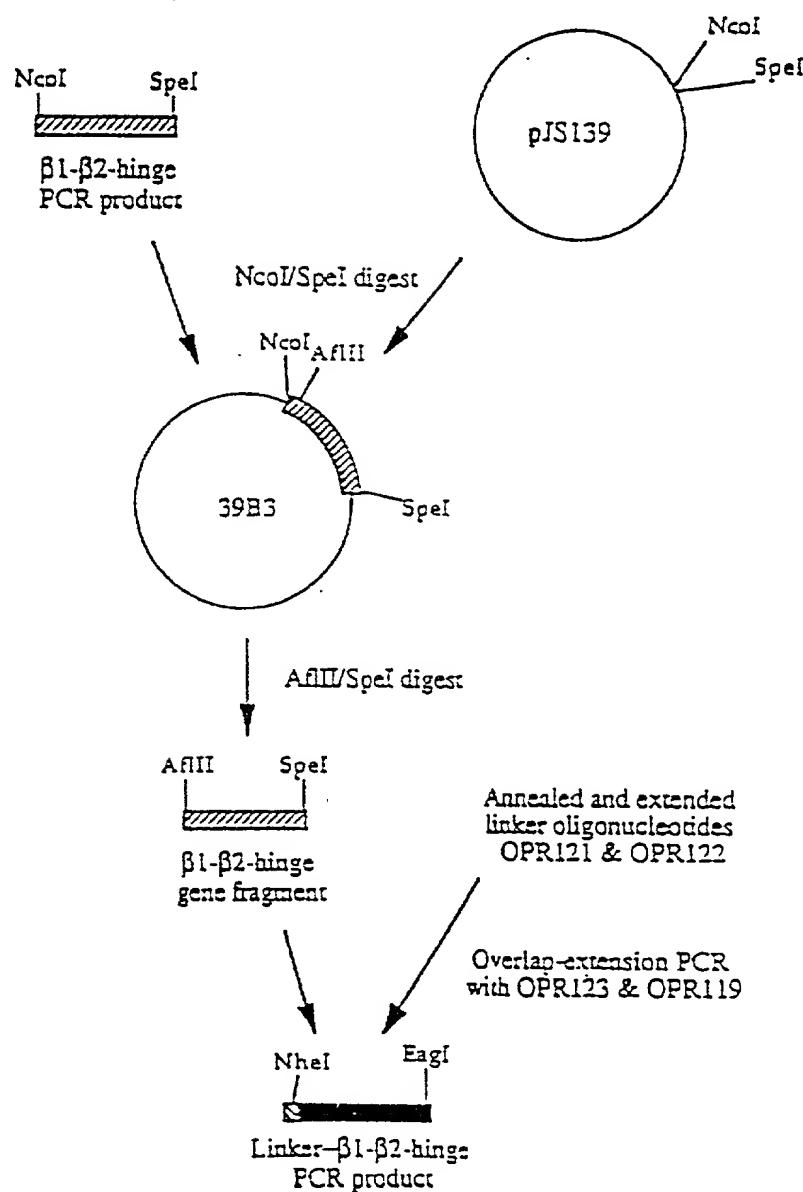


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Figure 5: HLA-DR1  $\beta$  chain cloning scheme

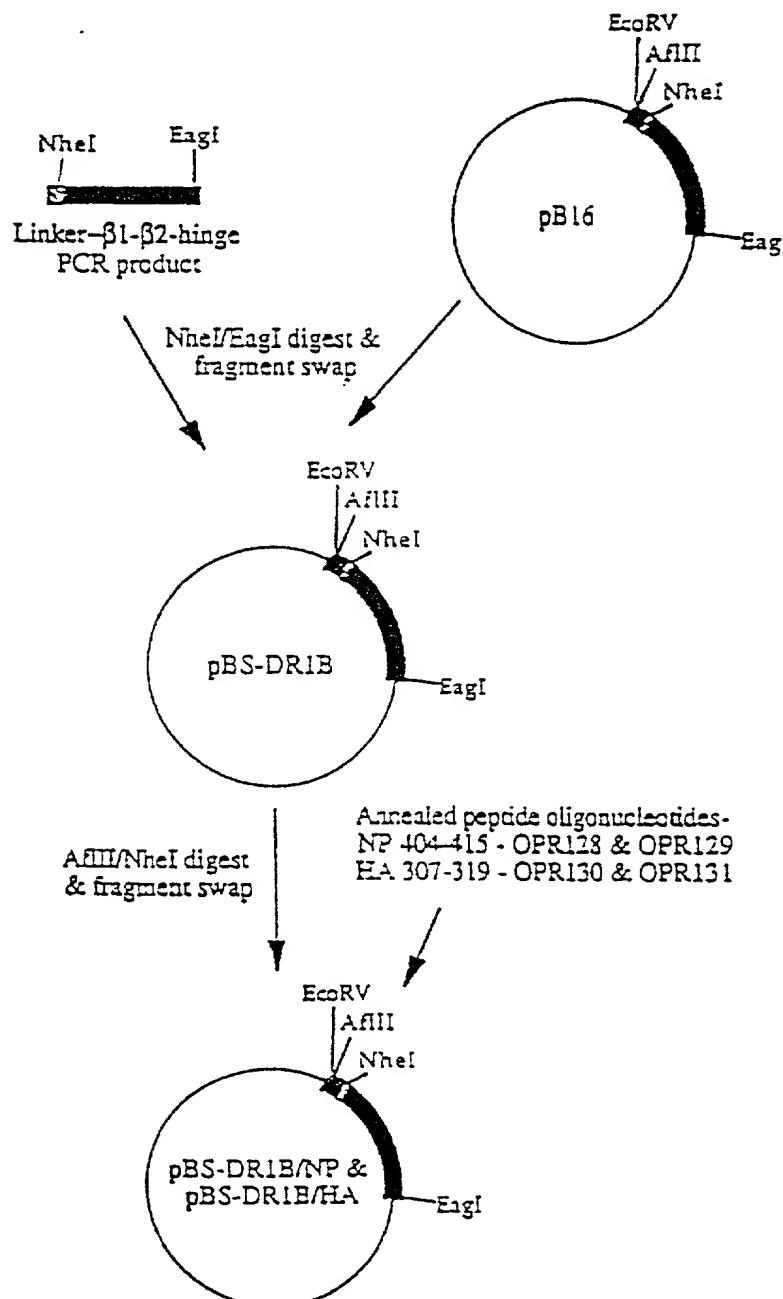
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Figure 5: cont.



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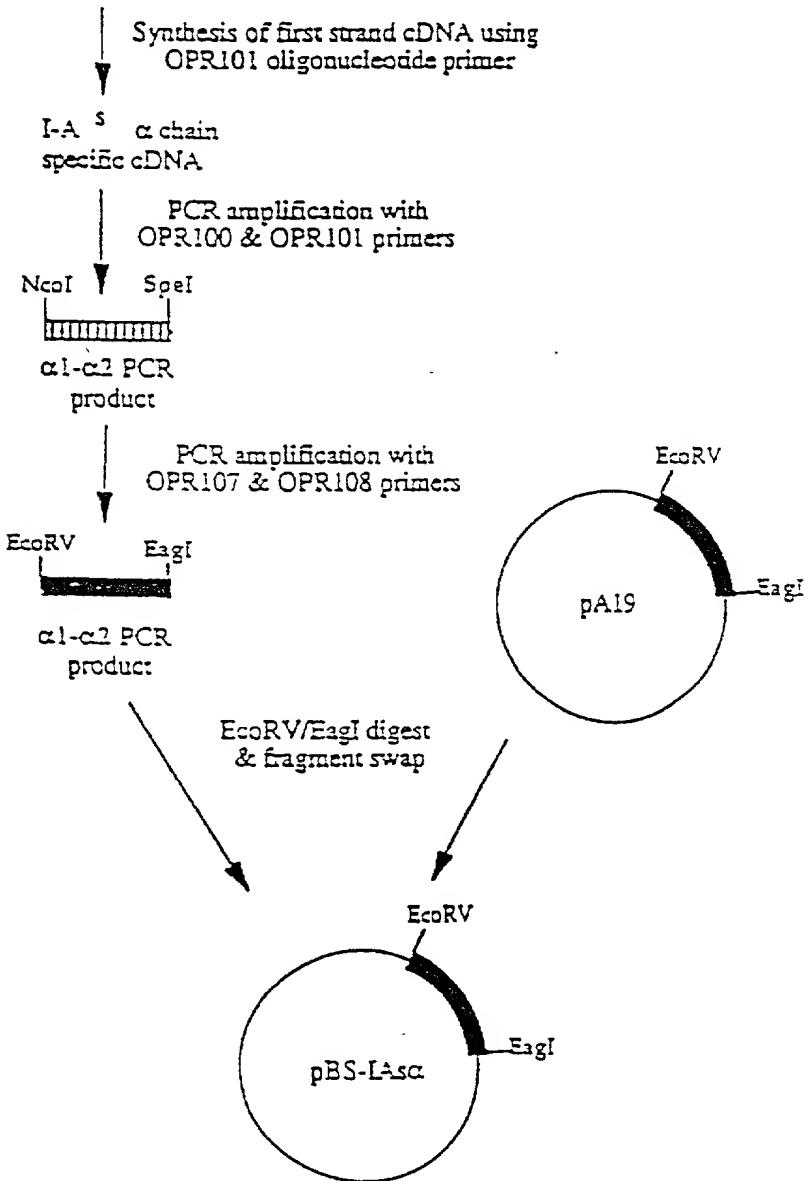
Figure 5: cont.



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Figure 6: I-A<sup>S</sup>  $\alpha$  chain cloning scheme

Total RNA isolated from  
SJL mouse spleen cells



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Figure 7: I-A<sup>s</sup>  $\beta$  chain cloning scheme

Total RNA isolated from  
SJL mouse spleen cells

↓  
Synthesis of first strand cDNA using  
OPR106 primer  
I-A<sup>s</sup>  $\beta$  specific  
cDNA template

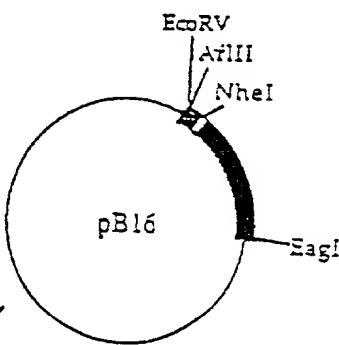
↓  
PCR amplification with  
VW310 & OPR106 primers

$\beta$ 1- $\beta$ 2 PCR product

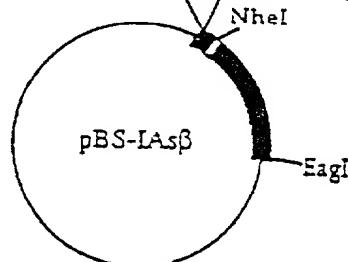
↓  
PCR amplification with  
VW309 & OPR106 primers

NheI      EagI  
Linker- $\beta$ 1- $\beta$ 2  
PCR product

NheI/EagI digest &  
fragment swap

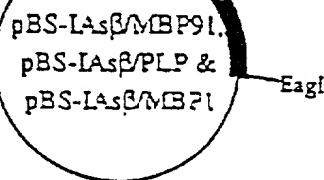


NheI/EagI  
digest



Annealed peptide oligonucleotides-  
MBP 91-103 - VW315 & VW316  
PLP 139-151 - VW313 & VW314  
MBP 1-14 - VW317 & VW318

AfIII/NheI digest  
& fragment swap



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## Figure 8 - Oligonucleotides used in constructing MHC vectors

I-A<sup>d</sup>/I-A<sup>s</sup> PCR primers and cloning oligonucleotides (restriction site are underlined).

OPR100

5'-GGG GGG GCC ATG GCC GAA GAC GAC ATT GAG GCC GAC-3'

OPR101

5'-GGG GGG ACT AGT CCA GTG TTT CAG AAC CGG CTC-3'

OPR107

5'-CCC CCC GAT ATC TCA GCT TCC AGC AGT GGA GAC GAC ATT GAG  
GCC G-3'

OPR108

5'-CCC CCC CGG CCG TCA CTT AGG TTT CCA GTG TTT CAG AAC CGG  
C-3'

OPR102

5'-GGG GGG ATG GCC GGA AAC TCC GAA AGG CAA CTC G-3'

OPR104

5'-GGG GGG ACT AGT CCA CTC CRC AGT GAT GGG GC-3'

OPR106

5'-CCC CCC CGG CCG TAC CTG AGG ACC ACT CCA CAG TCA TGG-3'

OPR112

5'-CCC CCC GAT ATC ACA GGT GTC TTA AGT GCT AGC GCA GGG GGC  
GCA AGC GGC GGA GGG GGA AAC TCC GAA AGG CAA TTC TTC-3'

OPR119

5'-AGC TTG ACA TCA CGG GTG TCT TAA GTG GAG-3'

OPR120-2

5'-CTA GCT CCA CTT AAG ACA CCT GTG ATA TCA-3'

VW310

5'-TCC GCA GGC GGC GGA GAC TCC GAA AGG CAA TTC G-3'

VW309

5'-CGA TCG CTA CGG CGG GTG GTG GTT CGG GTG GCG GCG GAG-3'

OPR136

5'-CCC CCC AGG CTT CCC GGG CCA CCA TCC CGT GCA GCA GAG CTC  
TG-3'

OPR139

5'-CCC CCC GAG CTC GAA TTC TCA TAA AGG CCC TGG GTG TCT G-3'

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## Figure 3 - cont

OPR132

5'-CCC CCC AAG CTT CCC GGG CCA CCA TGG CTC TGC AGA TCC CCA  
GC-3'

OPR133

5'-CCC CCC ACT TTA GGT CCT TGG GCT GGT CAG CAC C-3'

OPR134

5'-CCC CCC CCA TCA CTG TGG AGT GGA GGG-3'

OPR135

5'-CCC CCC GAG CTC GAA TTC TCA CTG CAG GAG CCC TGC TGG-3'

HLA-DRI PCR primers and cloning oligonucleotides.

DR1A-F

5'-GGG GGG AAG CTT ATG ACC AAA GAA GAA CAT GTG ATC ATC-3'

DR1A-B

5'-GGG GGG GGA TCC GTT CTC TGT AGT CTC TGG GAG AGG-3'

DR1B-F

5'-GGG GGG AAG CTT ATG GGG GAC ACC CGA CCA CGT TTC TTG TGG  
CAG C-3'

AF-N

5'-GGG GGG GCC ATG GCG ATC AAA GAA GAA CAT GTG ATC ATC-3'

AB-S

5'-GCG GCG ACT AGT GTT CTC TGT AGT CTC TGG GAG AGG-3'

OPR124

5'-GGG GGG AAG CTT GAT ATC TCA GCT TCC AGC AGT AGT ACC AAA  
GAA GAA CAT GTG ATC-3'

OPR125

5'-GGG GGG CGG CCG CTA CTT AGC TTT CTC TGG GAG AGG GCT TGG  
AGC-3'

DR1B-B

5'-GCG GCG GGA TCC CTT GCT CTG TGC AGA TTC AGA CC-3'

BF-NN

5'-GGG GGG GCC ATG GCC GCA TCC GCT AGC GGG GAC ACC CGA CCA  
CGT TTC TTG-3'

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Figure 3 - cont.

BB-5

5'-GCG GCG ACT AGT CTT GCT CTG TGC AGA TTC AGA CCG-3'

OPR121

5'-GTT GTC TTA AGT GGA GCT AGC GGA GGG GGC GGG TCC GGA GGT  
GGT GGG GAC ACC CG-3'

OPR122

5'-GAA ATG ACA TTC AAA CTT CAG CTG CCA CAA GAA ACG TGG TCG  
GGT GTC CCC ACC ACC-3'

OPR123

5'-GGG GGG CGG CGG TAC CTG AGG ACT TGC TCT GTG CAG ATT CAG-  
3'

Peptide oligonucleotides.

Ova 323-332

OPR110

5'-TTC AGT ATC TCT CAG GCT GTT CAC GCT GCT CAC GCT GAA ATC  
AAC GAA GCT GGT CGT G-3'

OPR111

5'-CTA GCA CGA CCA GCT TCG TTG ATT TCA GCC TGA GCA GCG TGA  
ACA GCC TGA GAG ATA C-3'

Ova H331R

OPR115

5'-TTC AGT ATC TCT CAG GCT GTT CAC GCT GCT CGG GCT GAA ATC  
AAC GAA GCT GGT CGT G-3'

OPR116

5'-CTA GCA CGA CCA GCT TCG TTG ATT TCA GCC CGA GCA GCG TGA  
ACA GCC TGA GAG ATA C-3'

Ova A332Y

OPR117

5'-TTC AGT ATC TCT CAG GCT GTT CAC GCT GCT CAC TAC GAA ATC  
AAC GAA GCT GGT CGT G-3'

OPR118

5'-CTA GCA CGA CCA GCT TCG TTG ATT TCA TAC TCA GCA GCG TGA  
ACA GCC TGA GAG ATA C-3'

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Figure 3 - cont.

HEL 74-86

OPRI140

5'-TTA AGT AAC CTG TGC AAC ATC CCC TGC AGC GCC CTG CTG AGC  
TCC G-3'

OPRI141

5'-CTA GCG GAG CTC AGC AGG GCG CTG CAGGGGG ATG TTG CAC AGG  
TTA C-3'

NP 404-415

OPRI129

5'-TTA AGT CAG ATC AGC GTG CAG CCC GCC TTC AGC GTG CAG G-3'

OPRI129

5'-CTA GCC TGC ACG CTG AAG GCG GCC TGA ACG CTG ATC TGA C-3'

HA 307-319

OPRI130

5'-TTA AGT CCC AAG TAC GTG AAG CAG AAC ACC CTG AAG CTG GCC  
ACC G-3'

OPRI131

5'-CTA GCG GTG GCC AGC TTC AGG GTG TTC TGC TTC ACG TAC TTG  
GGA C-3'

MBP 91-103

VW315

5'-TTA AGT CAC TAT GGC TCC CTG CCG CAG AAG TCC CAG CAC GGG  
CGC G-3'

VW316

5'-CTA GCG CGC CCG TGC TGG GAC TTC TGC GGC AGG GAG CCA TAG  
TGA C-3'

PLP 139-151

VW313

5'-TTA CTT CAC TCC CTG GGC AAG TGG CTG GGC CAC CCC GAC AAG  
TTC G-3'

VW314

5'-CTA GCG AAC TTG TTC GGG TGG CCC AGC CAC TTG CCC AGG GAG  
TGA C-3'

Figure 8 - cont.

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MBP 1-14

VW317

5'-TTA AGT ATG GCA TCC CAG AAG CGC CCG TCC CAG CGC TCC AAG  
TAC CTG G-3'

VW316

5'-CTA GCC AGG TAC TTG GAG CGC TGG GAC GGG CGC TTC TGG GAT  
GCC ATA C-3'5'-TTA AGT ATG GCA TCC CAG AAG CGC CCG TCC CAG CGC TCC AAG  
TAC CTG G-3'

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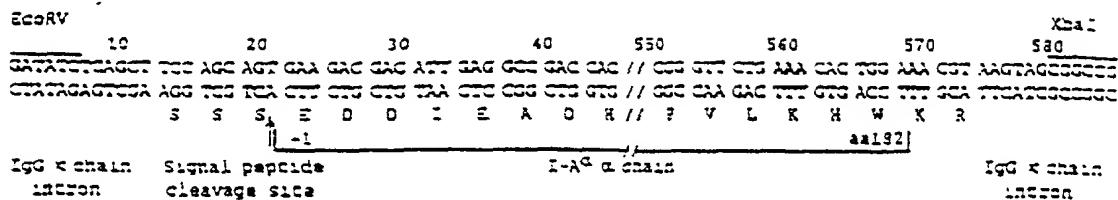
Figure 9A. Soluble I-A<sup>d</sup>  $\alpha$  chain construct

Figure 9B.

Soluble I-A<sup>d</sup>  $\beta$  chain construct

Restriction sites for insertion of  
oligonucleotides encoding peptides of interest

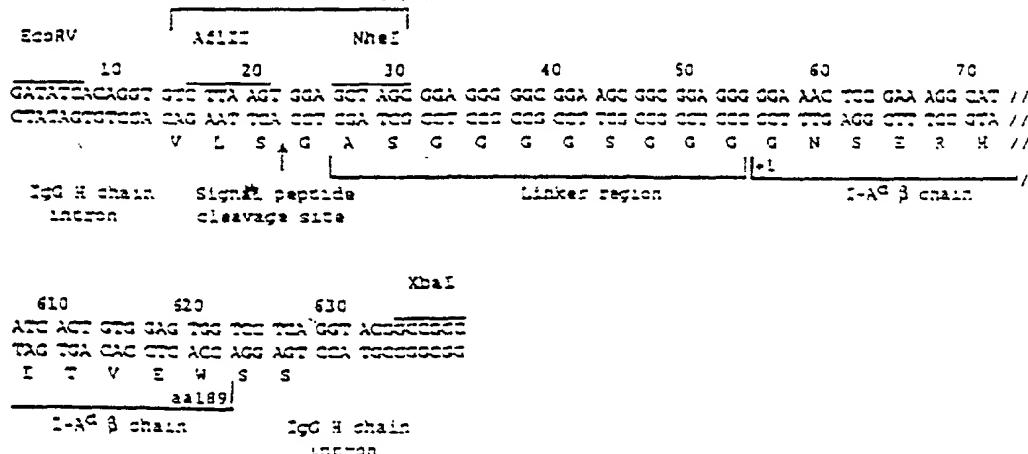


Figure 9C.

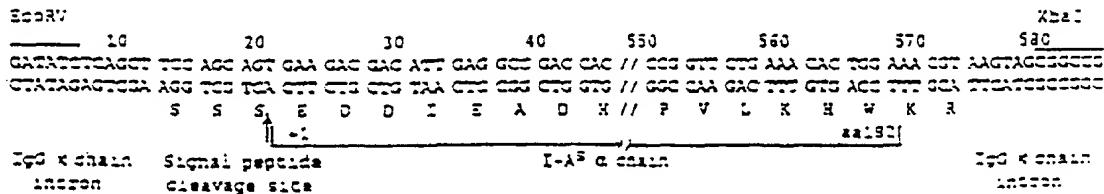
Soluble I-A<sup>s</sup>  $\alpha$  chain construct

Figure 9 - cont.

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Figure 9D. Soluble I-A<sup>5</sup>  $\beta$  chain construct

Restriction sites for insertion of oligonucleotides encoding peptides of interest

EcoRV	AflII	NheI					
10	20	30	40	50	60	70	
GATATTCGACGCT GTC TTA AGT GAA GCT AGC GCG GCT GGT GGT TCC GGT GGC GGC GGA GAC TCC GAA AGG CAT //	CTATATGCTTCA CAG TAT TCA GGT GAA TCC GCG CCA CCA CCA AGG CCA CCG CCG CCA AGG CTC AGG CTT TCC GTC //	V S G A S G G G S G G G G G D S E R H //					
				-1			
IgG H chain intron	Signal peptide cleavage site	Linker region				I- $A^{\delta}$ $\beta$ chain	

XbaI

610	620	630			
ATC ACT GTC GAG TGG TCC TCA GGT AC	GGGGGGGG				
TAG TCA CAC CTC ACC AGG ACT CCA	GGGGGGGG				
I T V E W S S					
aa139]					

I- $A^{\delta}$   $\beta$  chain      IgG H chain  
intron

Figure 9E. Soluble HLA-DR1 c chain construct

Diagram of the IgG kappa chain gene structure. The gene is 610 bp long, starting with EcoRV and ending with XbaI. It contains a signal peptide (bp 1-20), a cleavage site (bp 21-22), and the DR-1 kappa chain (bp 23-610). The sequence is shown with codons and a poly-A tail.

Figure 9F. Soluble HLA-DR1 B chain construct.

Restriction sites for insertion of oligonucleotides encoding peptides of interest									
EcoRV	AflII		NheI						
	10	20	30	40	50	60	70		
GATAATCAGAGCT GTC TTA AGT CGA CCT AGC GCA CGG CGG CGG TTC GGA GGT GGT GGG GAC ACC CGA CGA CGT TGC									
CTATAGCTTCTCA CAG AAT TCA CCT CGA TCG CCT CGG CGG CGG CGG CGT CGT CGA CGG CGT TGG CGT CGT CGA CGT CGA CGA									
V L S <b>↑</b> G A S G G G G S G G G C D T R ? R F									
IgG H chain	Signal peptide			Linker region				DR-1 $\beta$ chain	
intron	cleavage sites								
XbaI									
80	90	630	640	650	660				
TTC TGC CAG CGC AAG // TGT GAA TGT CGA CGC AGC AAG TCC TCA CGT CGC CGG CGG									
GGC ACT GTC GAC TTC // AGA CCT AGA CGT GTC TGG TGC AGC AGT CGT CGC CGG CGG									
L W Q L K // S E S A Q S K S S									
<b>AflII site pt. mutation</b>					aa198]				
DR-1 $\beta$ chain					IgG H chain				
					intron				

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Figure 10. Original Mammalian Cell Expression Vectors

Figure 10A

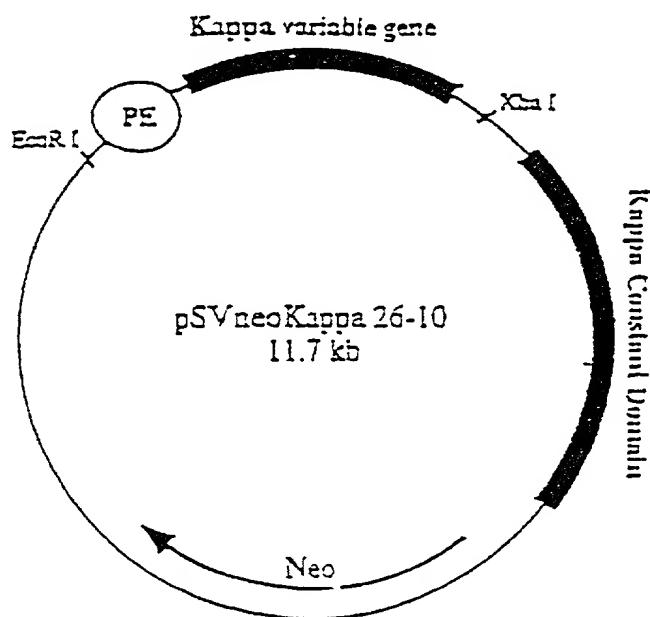
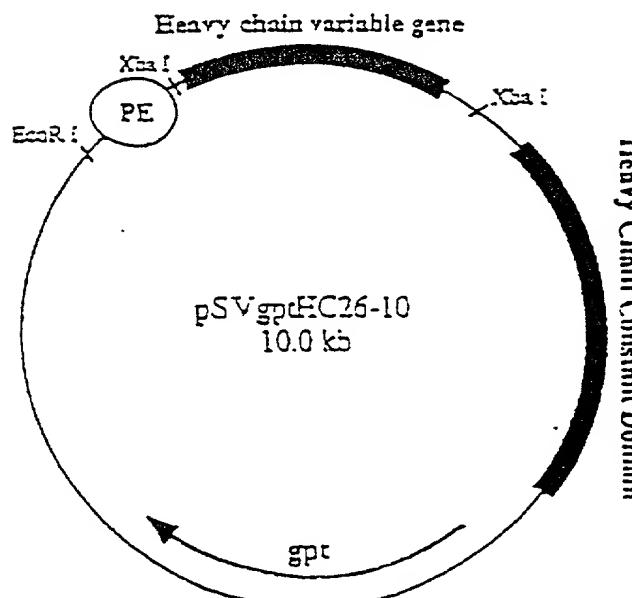


Figure 10B



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Figure 11. The 2.7 Kd Kappa and the 1.7 Kd Heavy Chain EcoR V and Eco I Mutated Constructs

Figure 11A

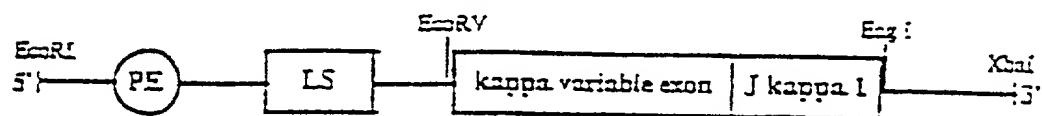
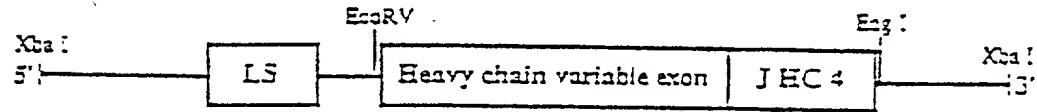
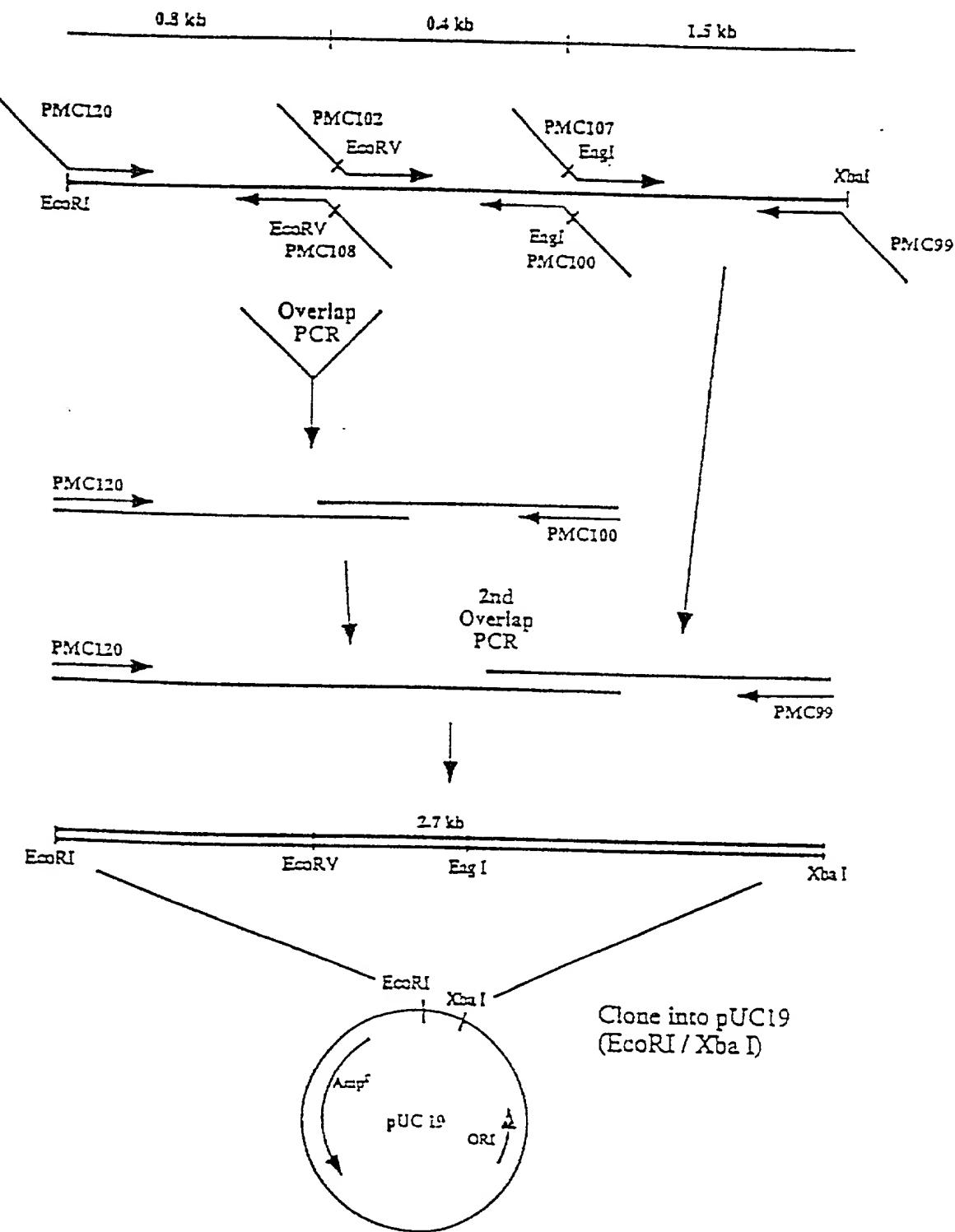


Figure 11B



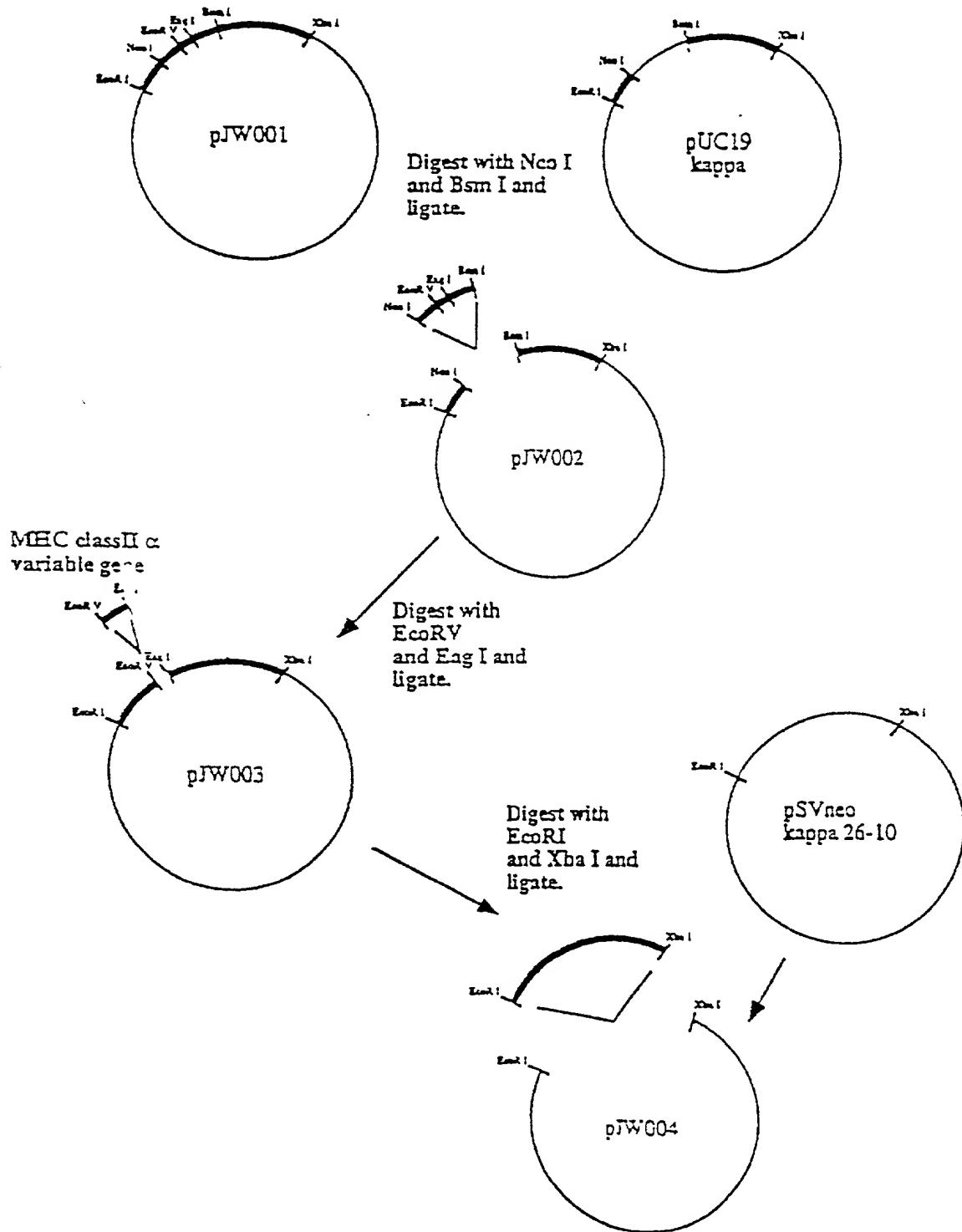
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Figure 12. PCR Site Directed Mutagenesis for Introducing EcoRV and EngI Restriction Sites into Kappa Chain 2.7kb Insert



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Figure 13. STRATEGY FOR CONSTRUCTING MHC CLASS II $\alpha$  / KAPPA CONSTANT GENE IN MAMMALIAN CELL EXPRESSION VECTOR

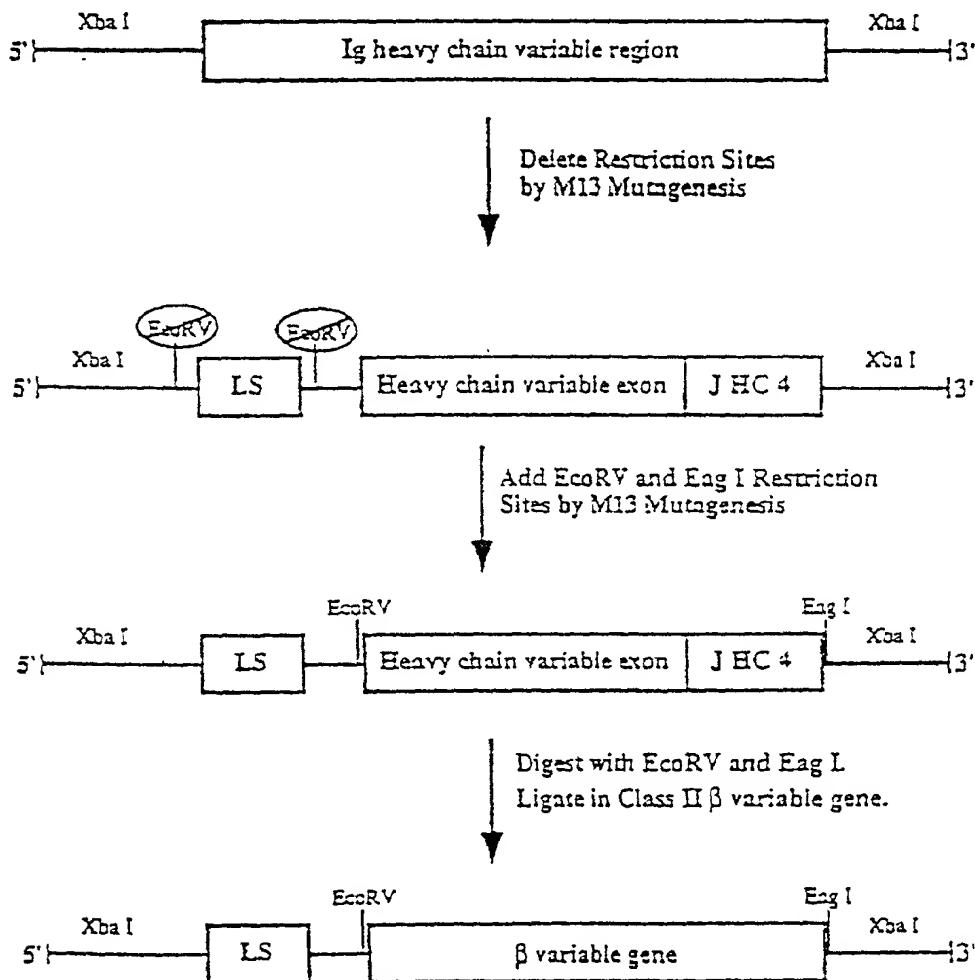


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Figure 14. Primers used for Sequencing Mutated 2.7 Kb Fragment

<u>Primer List</u>	<u>Sequence</u>
PMC-33	[5'GCTCAGCTGCTTGTTCAGTACTGATG']
PMC-77	[5'GTAAGTAGCGGCCG3']
PMC-111	[5'GGTATGTAAAAATAAACATCACAG3']
PMC-114	[5'GCTTGGCTACGGAGTTACTG']

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Figure 15. Strategy for M13 Mutagenesis and Cloning of the MEC II  $\beta$  Variable Gene

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Figure 16. Final Vectors for Expressing MHC II / Ig Chimeric Proteins

Figure 16A

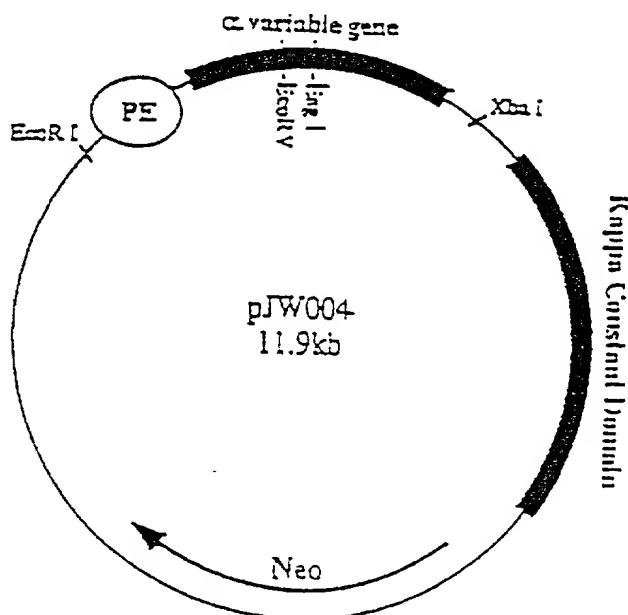
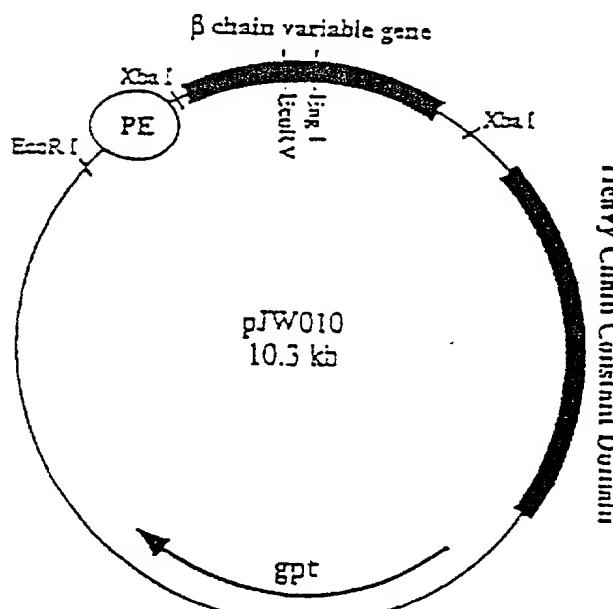
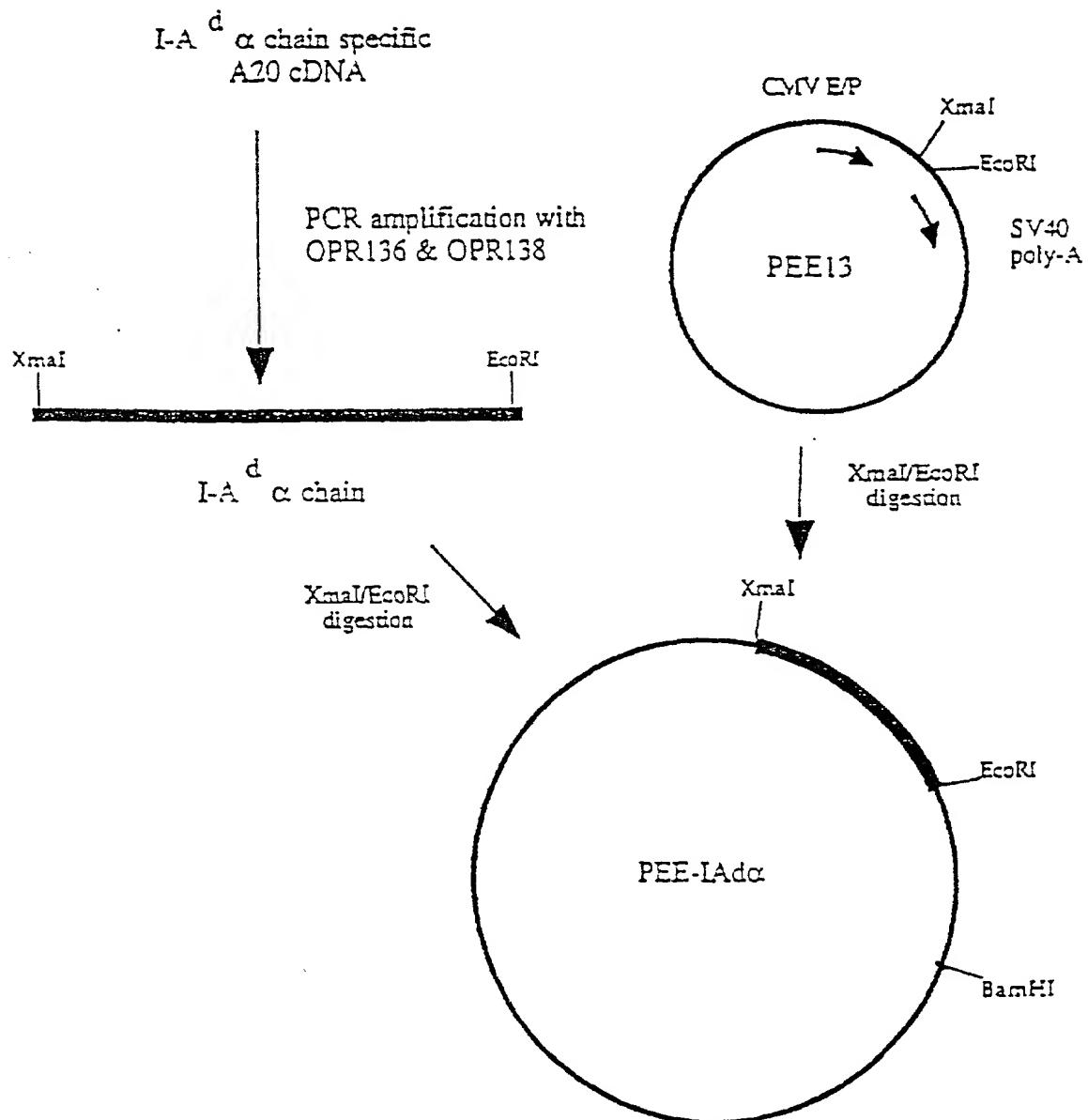


Figure 16B



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Figure 17 - Full length peptide linked MHC expression vectors



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Figure 17 - cont.

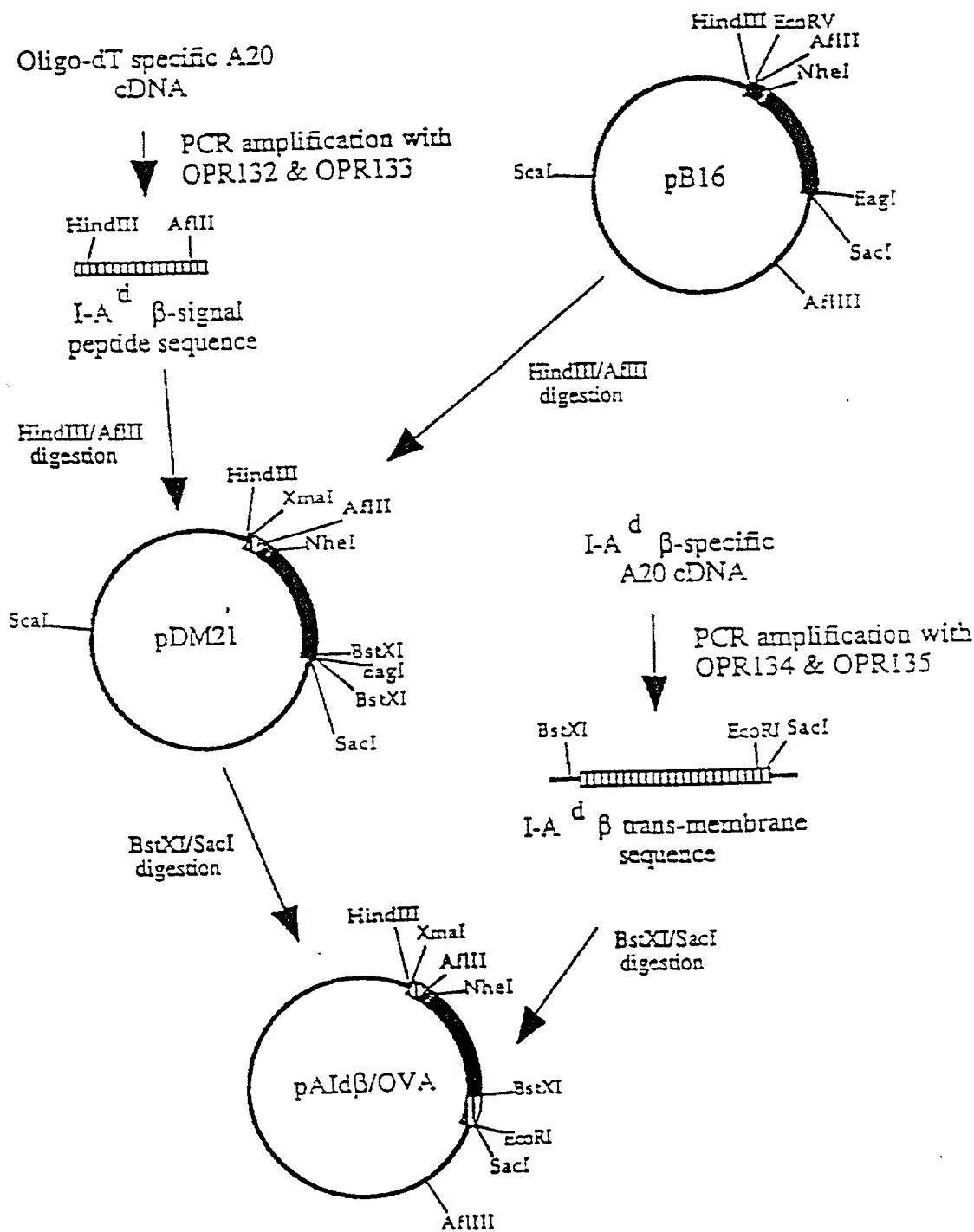


Figure 17 - cont.

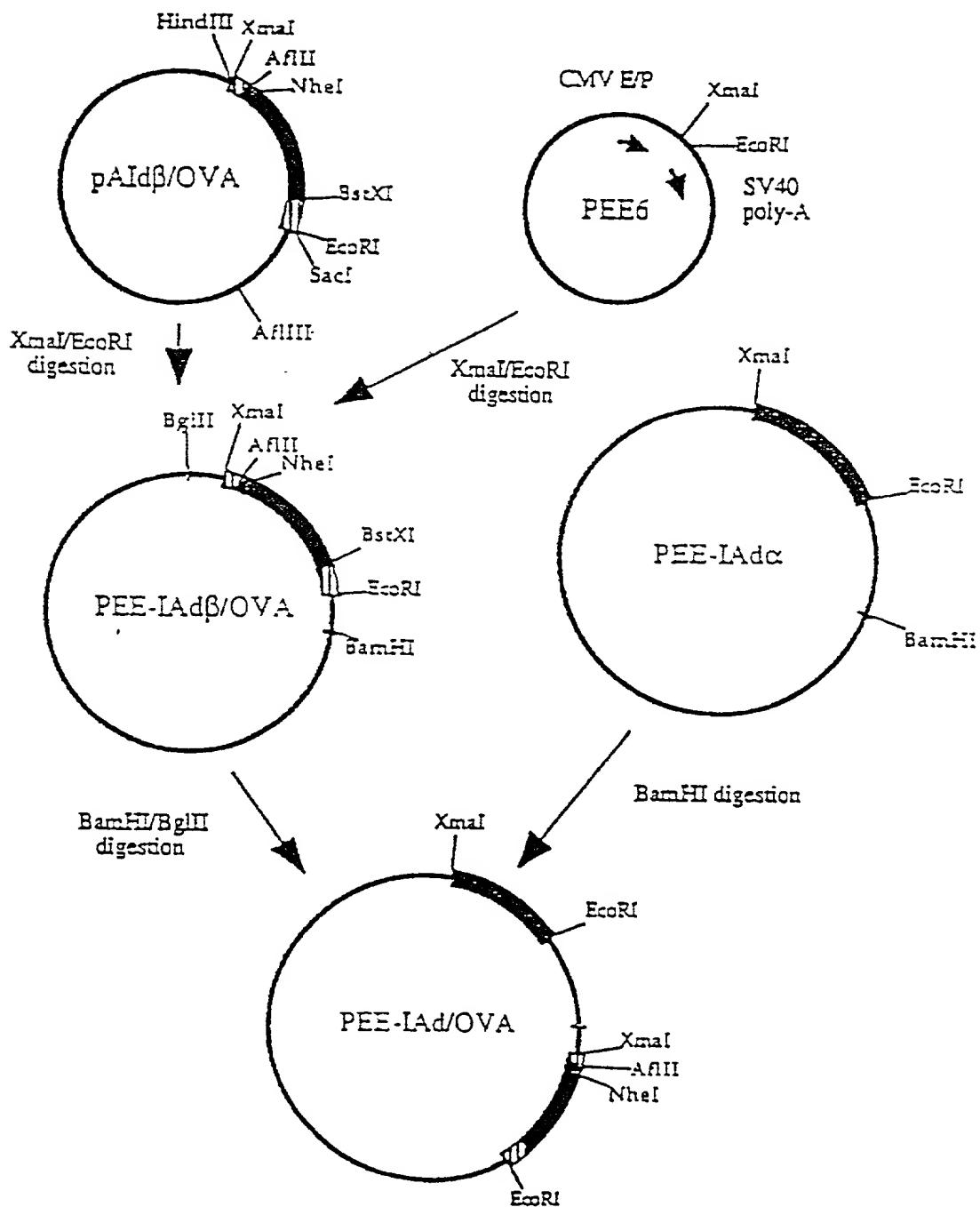


Figure 18A. Full length I-Ad  $\alpha$  chain insert

XbaI 10 20 30 40 50  
 CCGGAGGAGC A ATG CCG TCC AGC AGA CCT CTC ATG CTC CCG CTC CTC CCT GCG  
 GCGGAGGAGC G TAC GCG AGC TCC TGT GCA GAG TAA GAC CCT CTC CTC CAG CCT  
 H ? C S R A L I L G V L A

Kozak consensus: H ? C S R A L I L G V L A

I-A<sup>d</sup>  $\alpha$  chain signal peptide: I-A<sup>d</sup>  $\alpha$  chain signal peptide

60 70 80 90  
 CCT AAC ACT ATG CCT AGC CCT TCC GCA CCT GAA GAC GAC ATT GAC //  
 GAC TTC TCC TAC CAG TCC GAG AGC CCT GCA CCT CCT CCT TCA CTC //  
 L H T N L S L C C G A Z O O I E //  
 ||-1

I-A<sup>d</sup>  $\alpha$  chain signal peptide Signal peptide  
 cleavage site

EcoRI 750 760 770 780  
 CGA TCA CCT GCG AGC TCC AGA CAC CCT CCT CCT CCT TCA TCA GAA TGC  
 CCT ACT GCA CCT CCT TCC AGC TCC CCT CCT CCT CCT CCT CCT CCT CCT  
 R S G G T S R H ? G P L \*

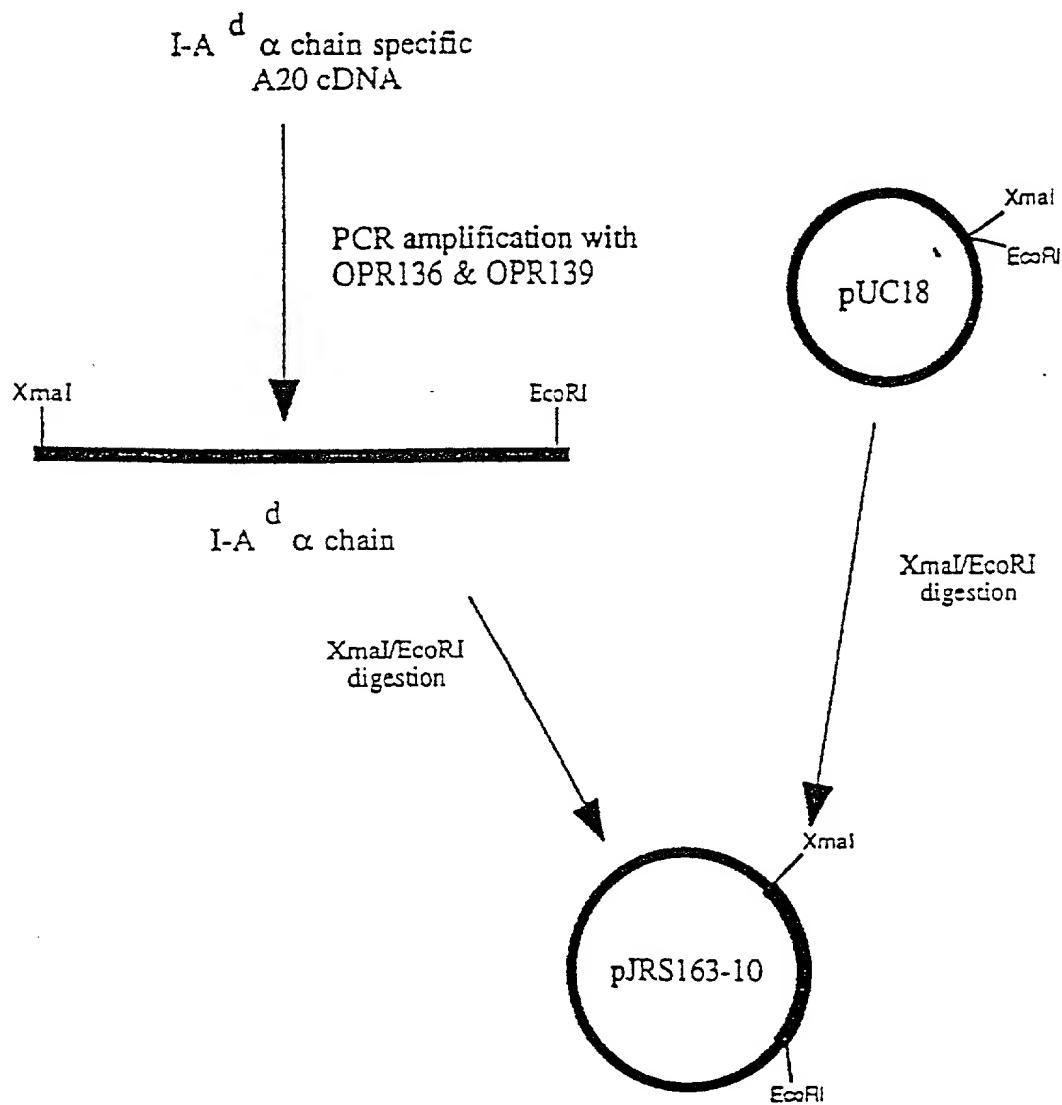
I-A<sup>d</sup>  $\alpha$  chain

Figure 18B. Full length L-Ad  $\beta$  chain insert

HindIII XbaI		10	20	30	40	50
A	G	C	T	G	T	A
A	A	G	T	C	G	T
T	T	A	G	T	C	A
T	T	A	G	T	C	A
		H	A	L	Q	I
		P	S	L	L	S
		A				
Kozak consensus		I-A <sup>d</sup> 3 chain signal peptide				
AFLII						
		60	70	80	90	100
G	C	T	G	T	A	T
C	T	G	T	C	G	T
C	A	G	C	A	C	A
C	A	G	C	A	G	T
A	V	V	V	L	H	V
				L	S	S
				S	S	P
				R	T	T
				S	A	I
I-A <sup>d</sup> 3 chain signal peptide				Signal peptide cleavage site		
		110	120	130	140	
T	T	C	G	C	T	
T	C	G	C	T	C	
T	C	G	C	T	C	
A	G	T	C	A	T	
A	G	T	C	A	T	
S	Q	A	V	K	A	H
				A	H	E
				T	I	N
				E	N	E
				A	G	R
Ova peptide						
		150	160	170	180	190
G	C	T	A	C	G	A
C	T	A	C	G	A	C
C	T	A	C	G	A	C
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
NheI						
		200	210	220	230	240
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
Linker region						
		250	260	270	280	290
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		300	310	320	330	340
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
ECORI SacI						
		350	360	370	380	390
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		400	410	420	430	440
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
ScaI						
		450	460	470	480	490
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		500	510	520	530	540
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
SstI						
		550	560	570	580	590
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		600	610	620	630	640
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		650	660	670	680	690
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		700	710	720	730	740
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		750	760	770	780	790
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		800	810	820	830	840
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		850	860	870	880	890
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		900	910	920	930	940
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		950	960	970	980	990
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	N
				N	S	E
				S	E	R
I-A <sup>d</sup> 3 chain						
		1000	1010	1020	1030	1040
C	C	T	A	T	C	T
C	C	T	A	T	C	T
C	C	T	A	T	C	T
A	T	T	C	T	C	T
A	T	T	C	T	C	T
A	S	C	C	C	C	C
				S	G	G
				G	G	

FIGURE 19 (Sheet 1 of 7)

## Full length peptide linked MHC expression vectors



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FIGURE 19 (Sheet 2 of 7)

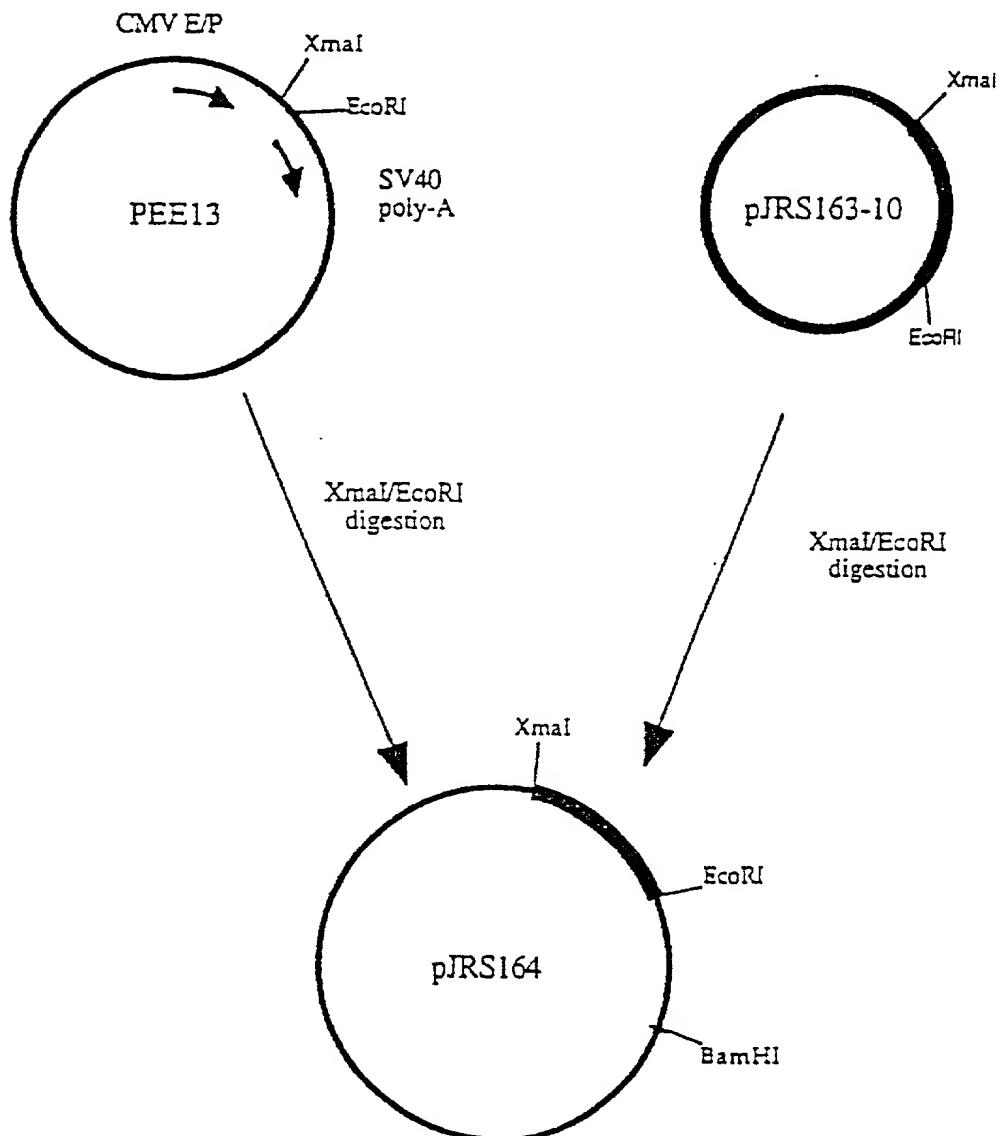
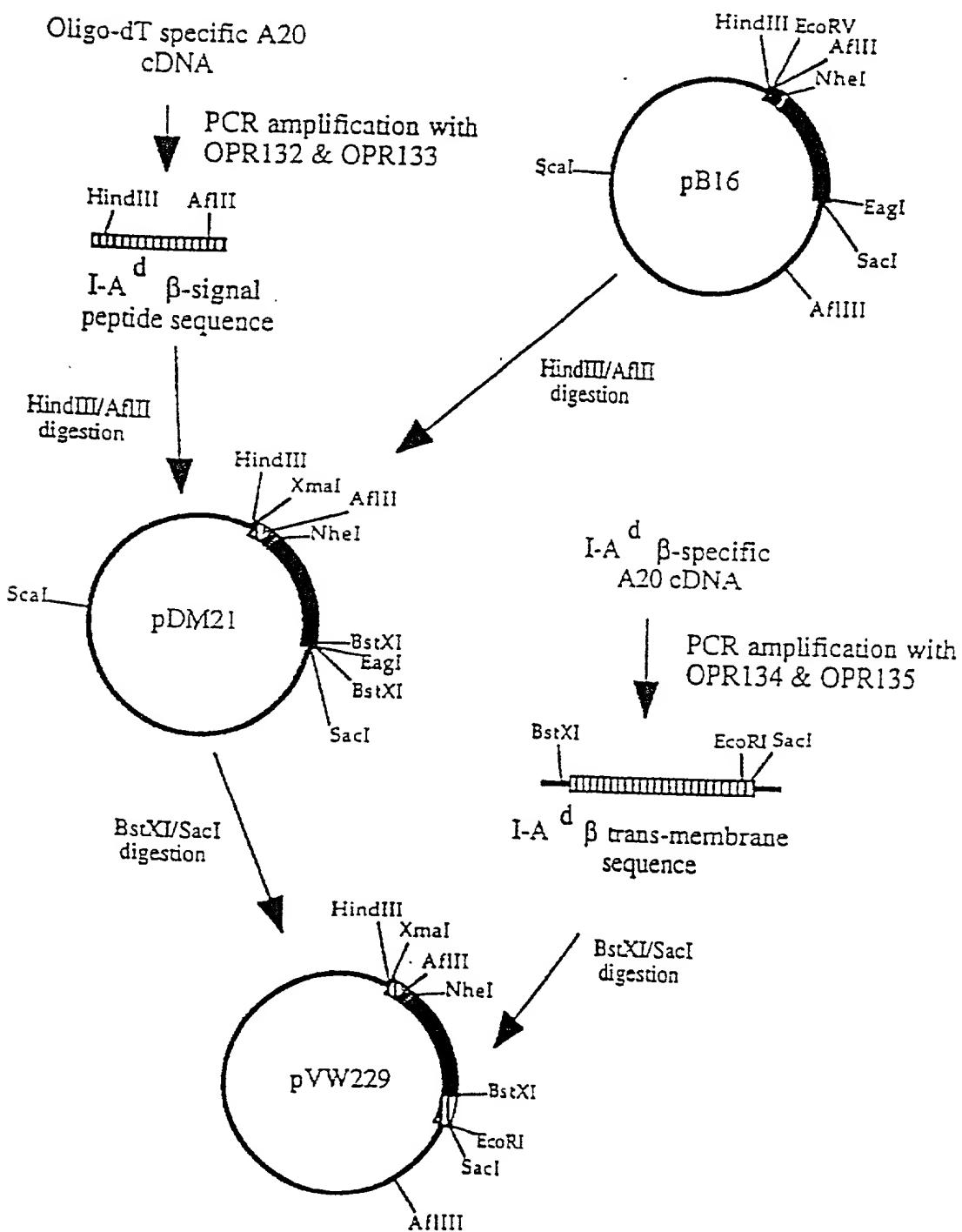
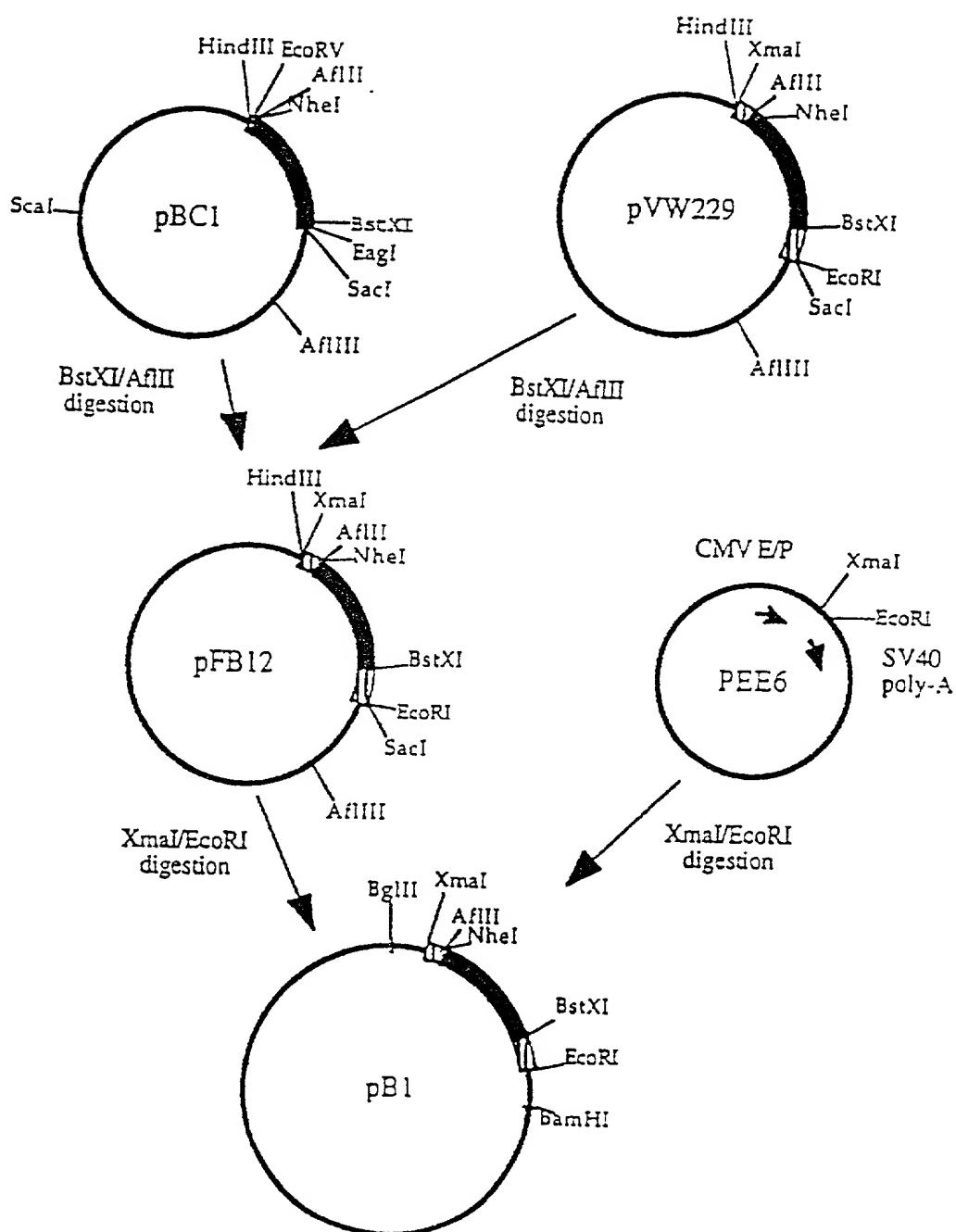


FIGURE 19 (Sheet 3 of 7)

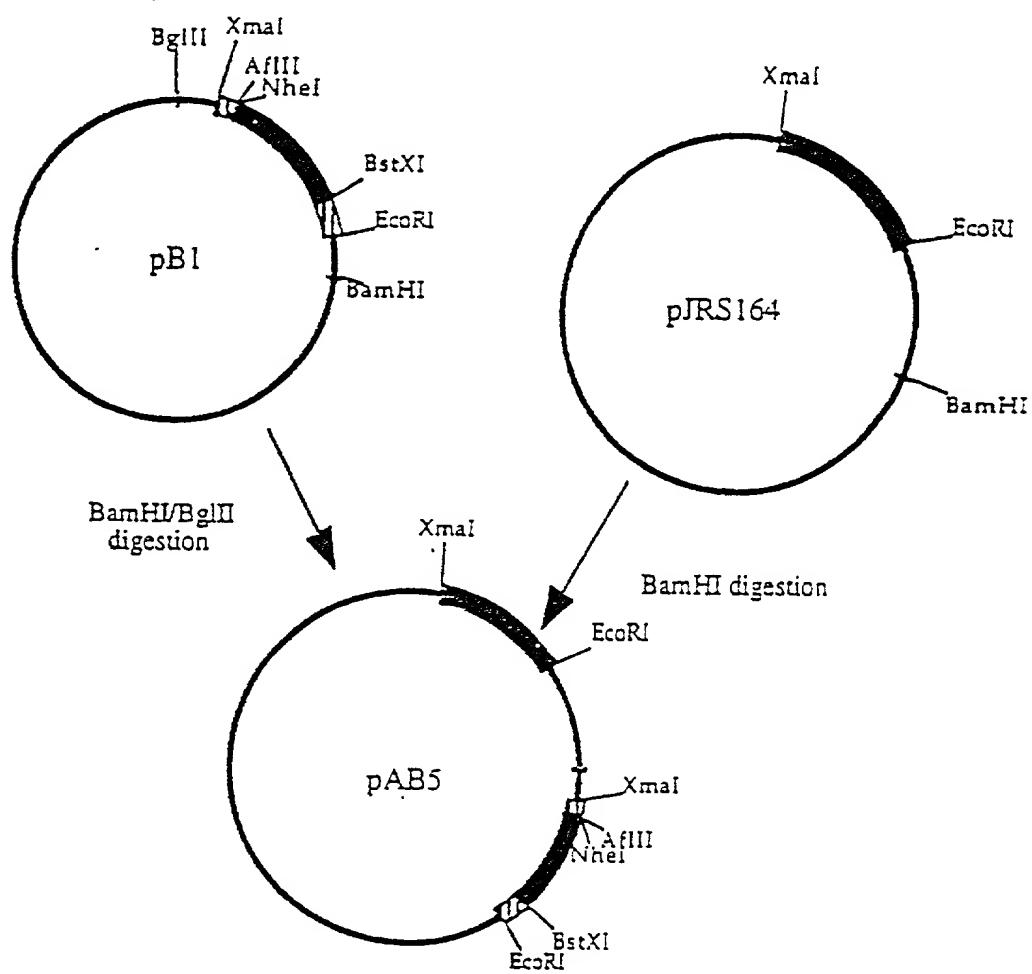


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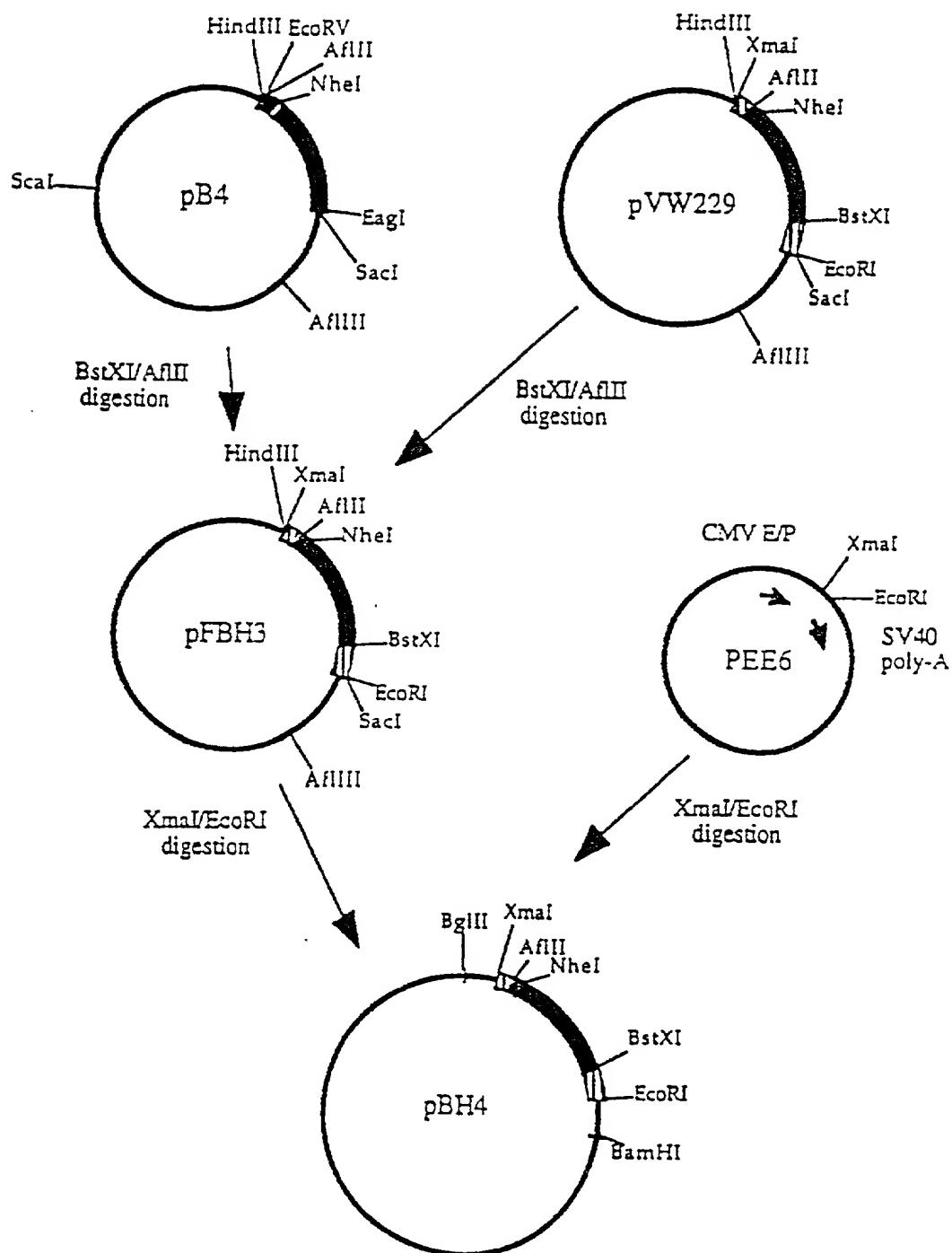
FIGURE 19 (Sheet 4 of 7)

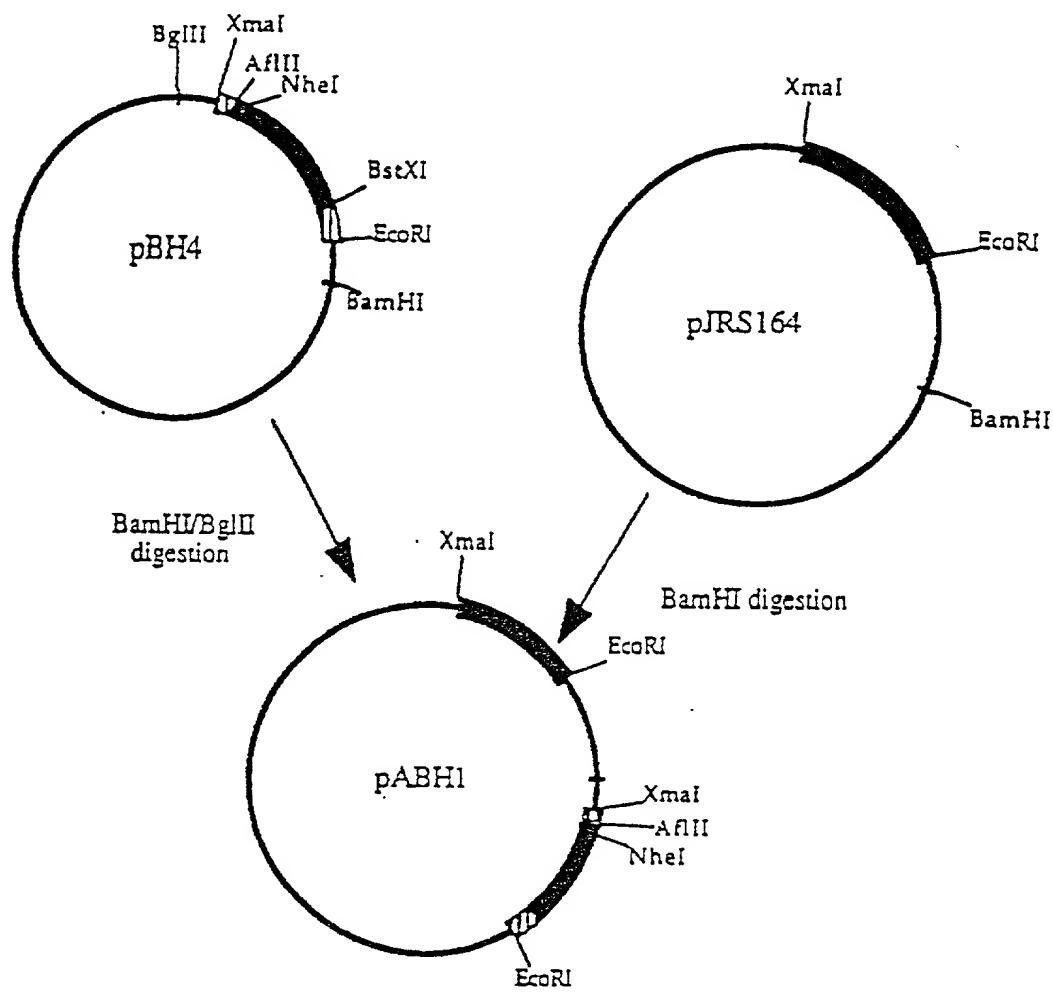


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FIGURE 19 (Sheet 5 of 7)



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FIGURE 19 (Sheet 6 of 7)



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FIGURE 19 (Sheet 7 of 7)

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FIGURE 20

## Oligonucleotides used in cloning

## OPR132

I-A<sup>d</sup>  $\beta$  signal peptide front primer with Kozak consensus for CellTech vector -

HindIII/XbaI sites

5'-CCC CCC AAG CTT CCC GGG CCA CCA TGG CTC TGC AGA TCC CCA  
GC-3'

## OPR133

I-A<sup>d</sup>  $\beta$  signal peptide back primer with Kozak consensus for CellTech vector - AflII site5'-CCC CCC ACT TAA GGT CCT TGG GCT GCT CAG CAC C-3'

## OPR134

I-A<sup>d</sup>  $\beta$  transmembrane front primer for CellTech vector - BstXI sites5'-CCC CCC CCA TCA CTG TGG AGT GGA GGG-3'

## OPR135

I-A<sup>d</sup>  $\beta$  transmembrane back primer for CellTech vector - SstI, EcoRI sites5'-CCC CCC GAG CTC GAA TCC TCA CTG CAG GAG CCC TGC TGG-3'

## OPR136

I-A<sup>d</sup>  $\alpha$  signal peptide front primer with Kozak consensus for CellTech vector -

HindIII/XbaI sites

5'-CCC CCC AAG CTT CCC GGG CCA CCA TGC CGT GCA GCA GAG CTC  
TG-3'

## OPR139

I-A<sup>d</sup>  $\alpha$  transmembrane primer for CellTech vector - SstI/EcoRI sites5'-CCC CCC GAG CTC GAA TCC TCA TAA AGG CCC TGG GTG TCT G-3'

## B7-1-2F

Murine B7-1 front primer with Kozak consensus for CloneTech vector - NotI site

5'-CCC CCC CCG CGG CCG CCC CAC CAT GGG ACT GAG TAA CAT TCT  
C-3'

## B7-1-2B

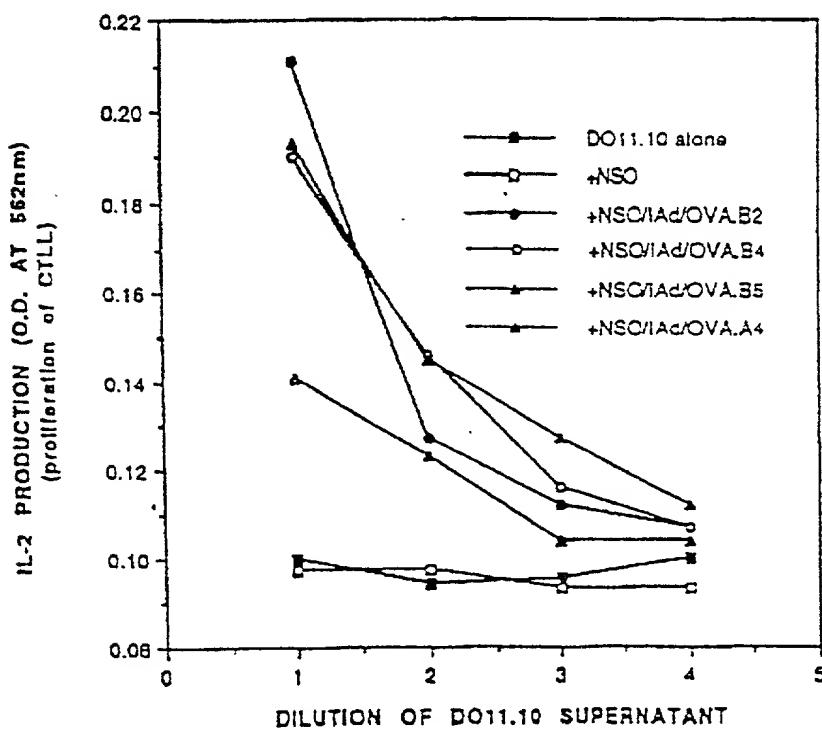
Murine B7-1 BACK primer for CloneTech vector - NotI site

5'-CCC CCC GCG GCC GCT TTA AAA ACA TGT ATC ACT TTT-3'

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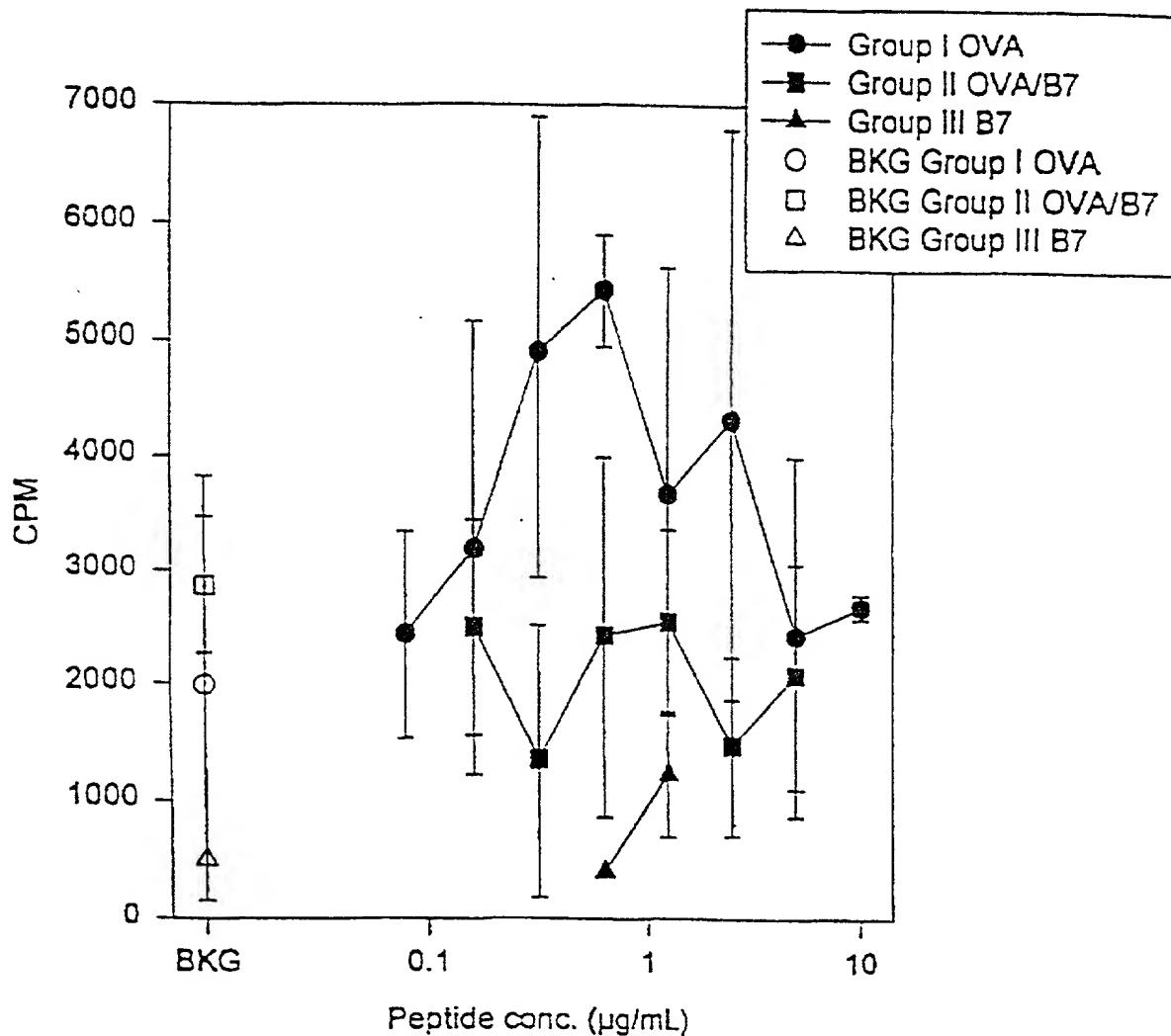
FIGURE 21

NSO/ClassII/OVA clones Stimulate IL-2 Production from DO11.10



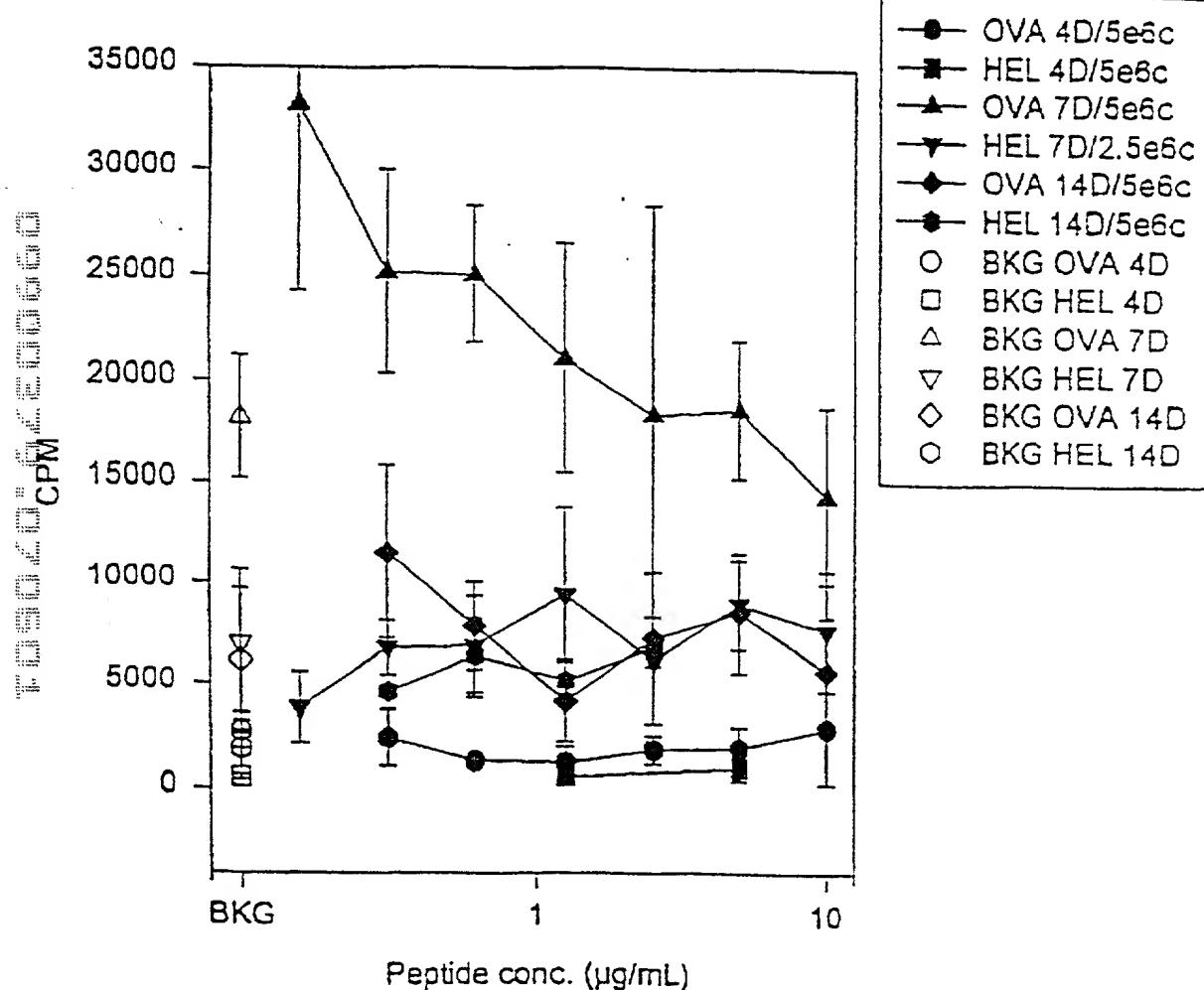
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FIGURE 22

### INTRAMUSCULAR IAd/OVA & B7 DNA INJECTIONS PROLIFERATION ASSAY



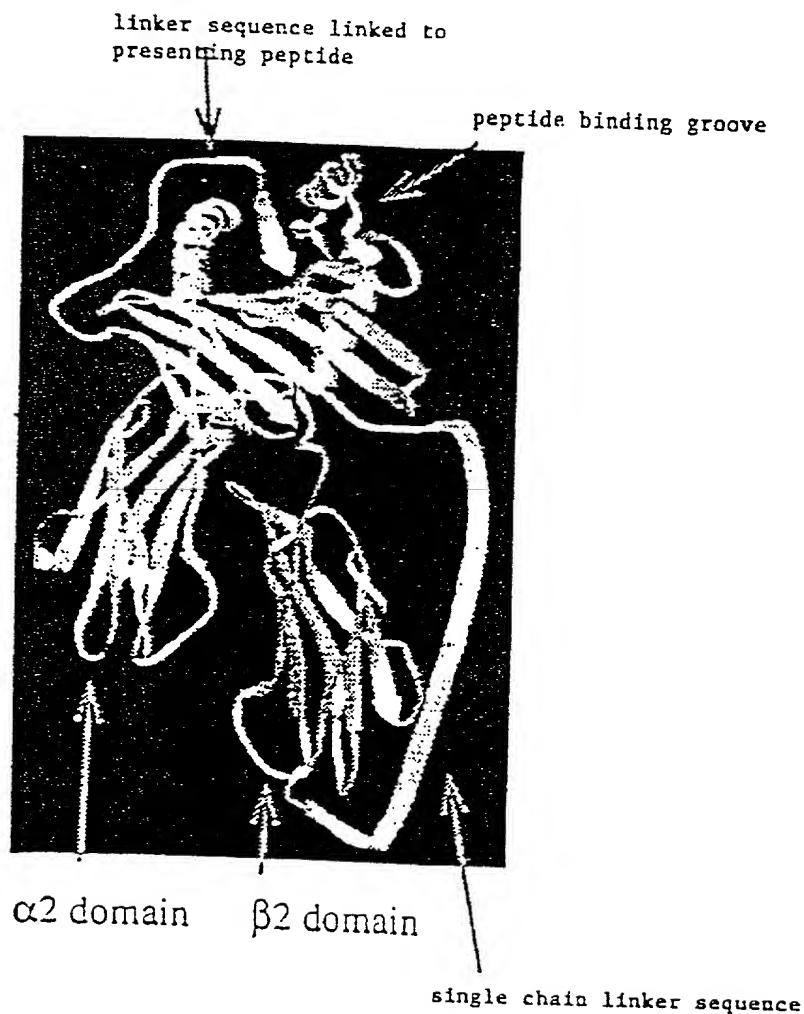
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FIGURE 23

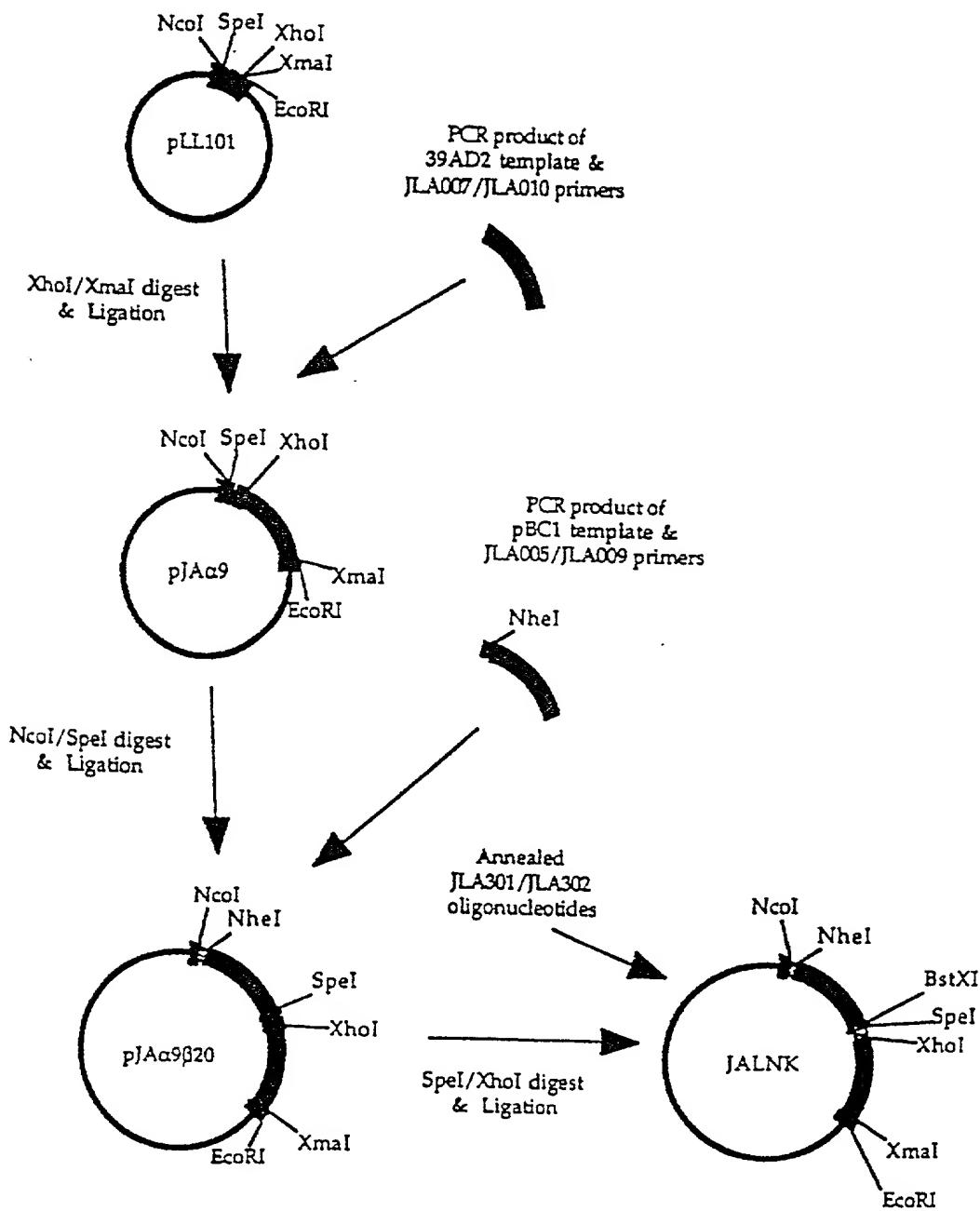
INTRADERMAL IA<sub>d</sub>/OVA & IA<sub>d</sub>/HEL DNA INJECTIONS  
PROLIFERATION ASSAY  
4, 7 & 14 DAYS POST INJECTION



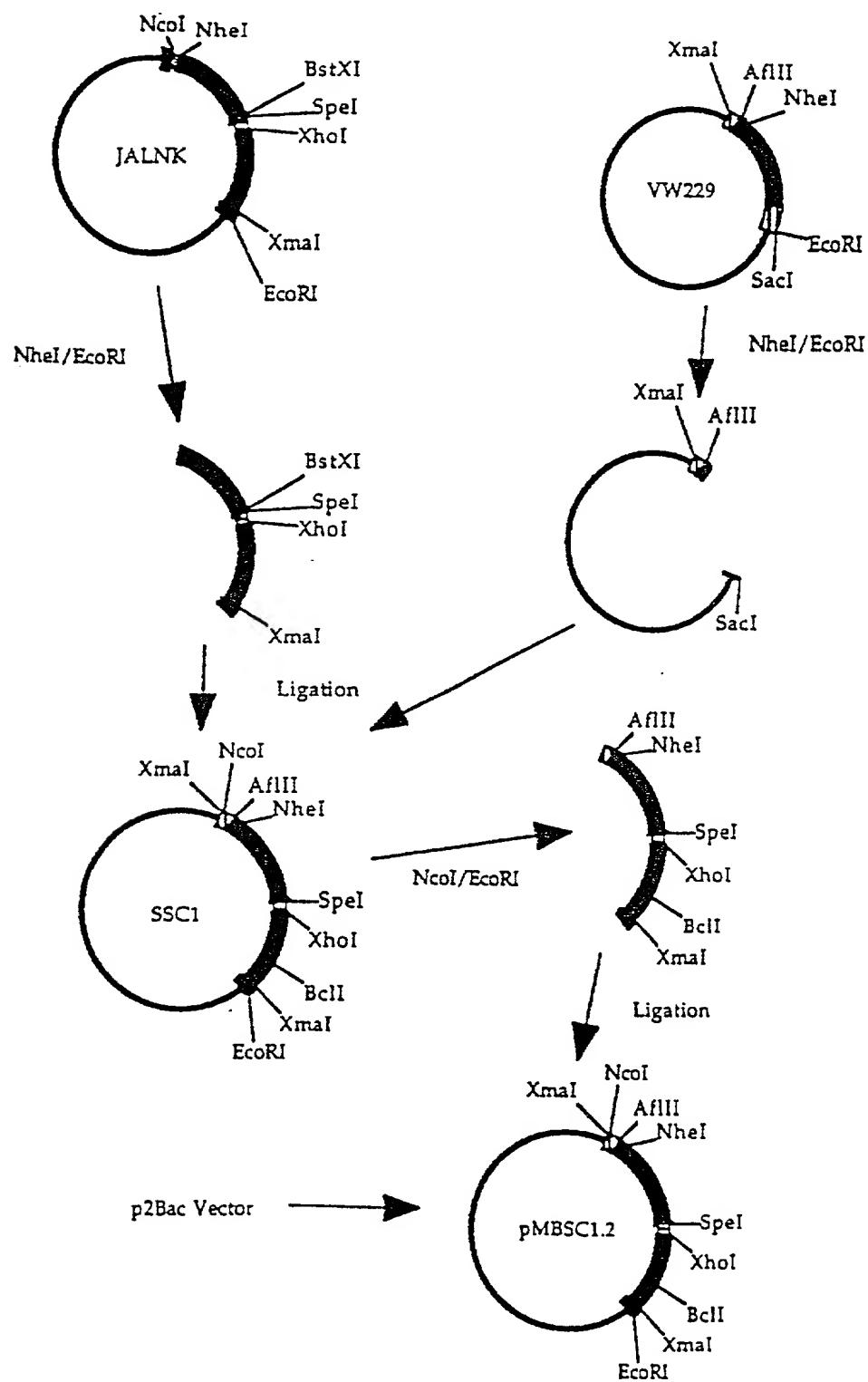
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FIGURE 24

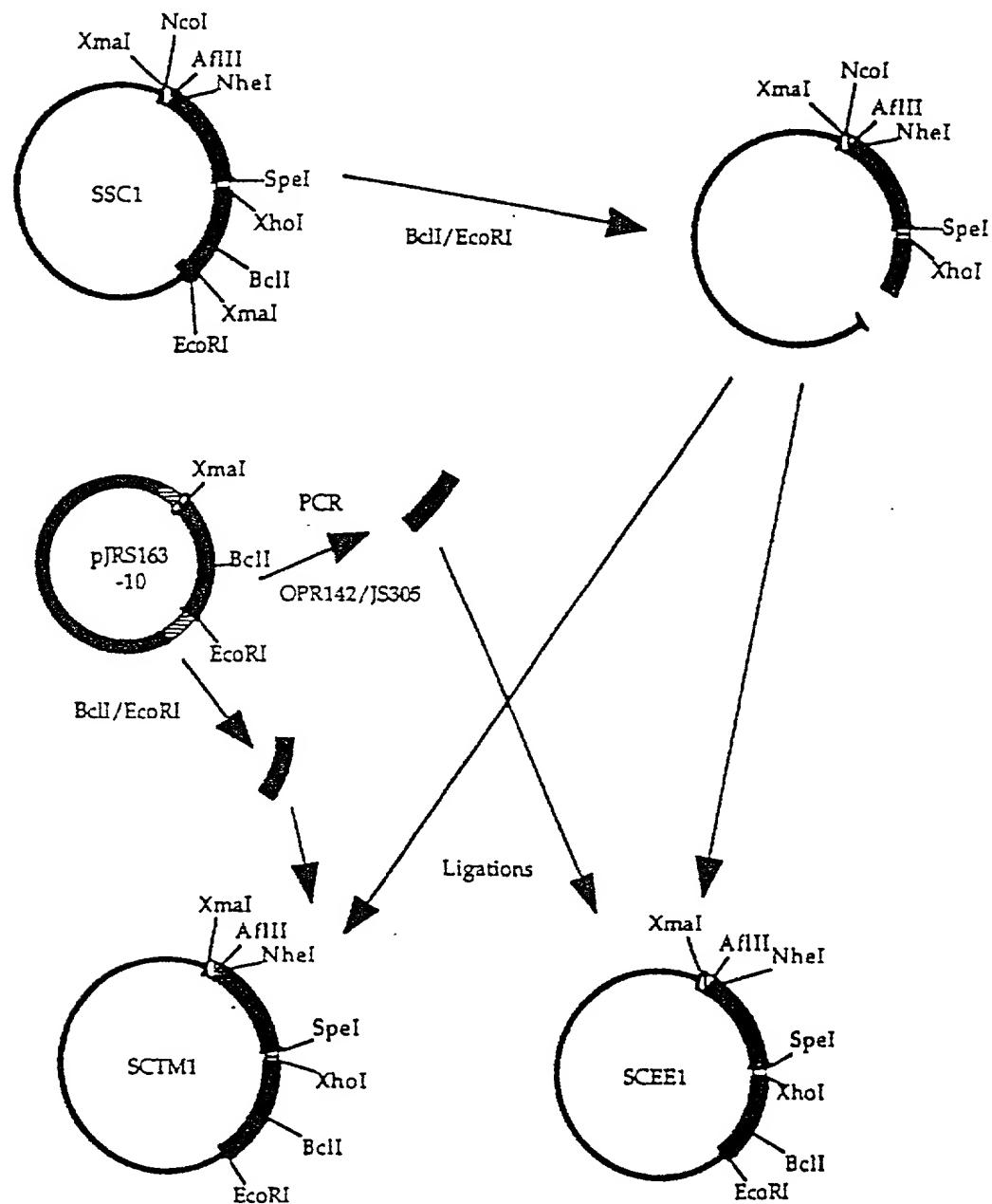


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FIGURE 25 (SHEET 1 OF 4)

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FIGURE 25 (SHEET 2 OF 4)

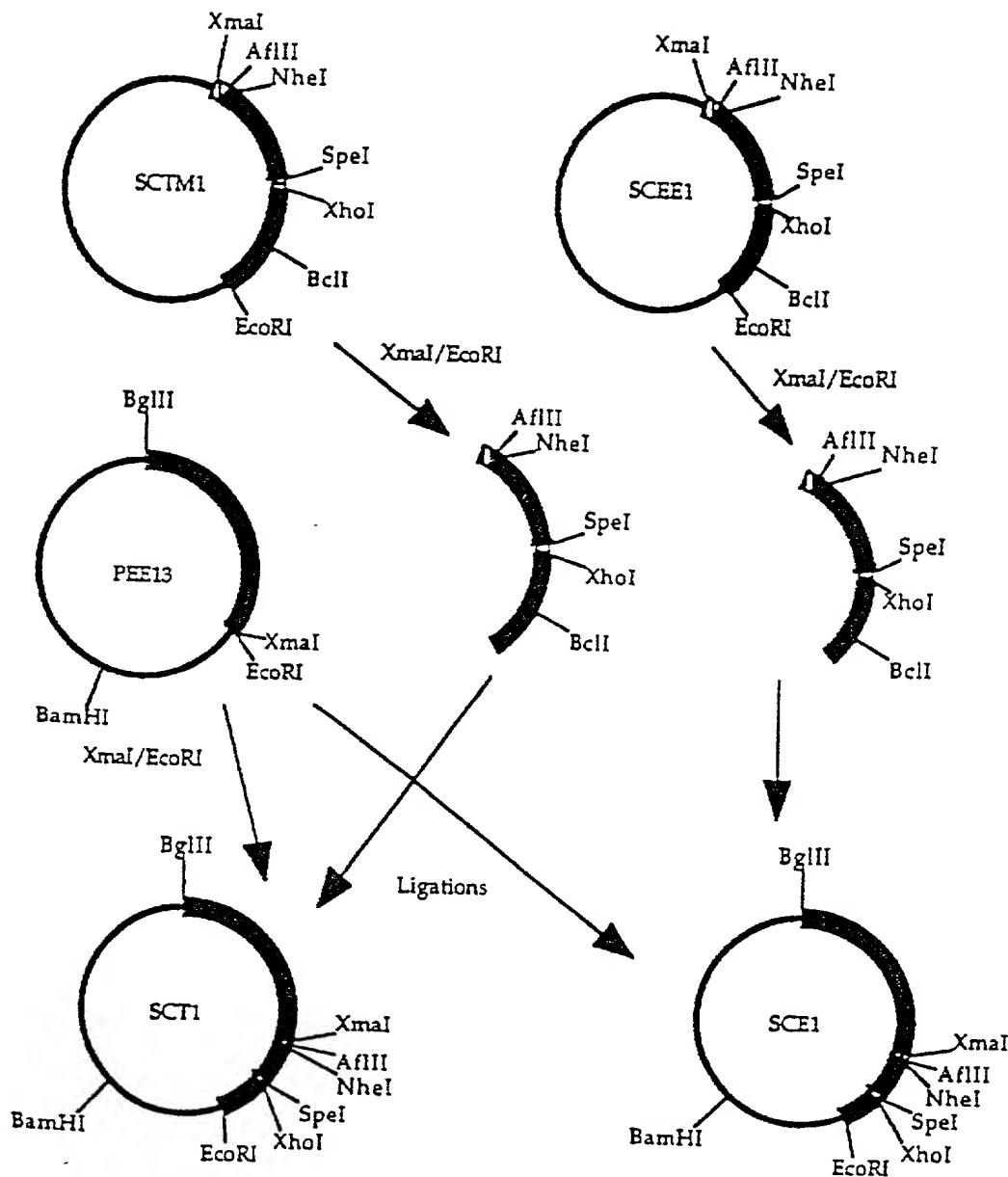


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FIGURE 25 (SHEET 3 OF 4)



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FIGURE 25 (SHEET 4 OF 4)



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FIGURE 26

## JLA-005

5'-CCCCCGCCATGGCCGCTAGCGGAGGGGGCGGAAGC-3'

## JLA-007

5'-CCCGGGGCCTCGAGTGAAGACGACATTGAGGCCGAC-3'

## JLA-009

5'-CCCCCCACTAGTCCACTCCACAGTGATGGGGCT-3'

## JLA-010

5'-CCCCCCCCCGGGACCAGTGTTCAGAACCGGCTCCTC-3'

## JLA-301

5'-TCGAGGAACCGCCACCGCCAGAACCGCCGCCACCGA-  
ACCACCAACCGCCGCTGCCACCGCCACCA-3'

## JLA-302

5'-CTAGTGGTGGCGGTGGCAGCGGCGGTGGTGGTCCGG-  
TGGCGCGGTTCTGGCGGTGGCGGTTCC-3'

## OPR-142

5'-CTTGGGAATCTTGACTAAGAGG-3'

## JS-305

5'-CAGGTCGAATTCTCATCCATGGCATGTAACCTTCTT-  
CCTCCCAGTGTTCAGAACCGG-3'

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FIGURE 27 (SHEET 1 OF 4)

10                    20                    30                    40                    50  
 \*                    \*                    \*                    \*                    \*  
 CCACC ATG GCT CTG CAG ATC CCC AGC CTC CTC CTC TCA GCT GCT GTG GTG  
 GGTGG TAC CGA GAC GTC TAG GGG TCG GAG GAG AGT CGA CGA CAC CAC  
 M A L Q I P S L L L S A A V V>  
 <----- I-Ad  $\beta$  chain leader ----->

60                    70                    80                    90  
 \*                    \*                    \*                    \*  
 GTG CTG ATG GTG CTG AGC AGC CCA AGC ACC TTA AGT ATC TCT CAG GCT  
 CAC GAC TAC CAC GAC TCG TCG GGT TCC TGG AAT TCA TAG AGA GTC CGA  
 V L M V L S S P R T L S I S Q A>  
 -----><-----

100                  110                  120                  130                  140  
 \*                    \*                    \*                    \*                    \*  
 GTT CAC GCT GCT CAC GCT GAA ATC AAC GAA GCT GGT CGT GCT ACC CGA  
 CAA GTG CGA CGA GTG CGA CTT TAG TTG CTT CGA CCA CGA CGA TCG CCT  
 V H A A H A E I N E A G R A S G>  
 ----- OVA 323-339 -----><-----

150                  160                  170                  180                  190  
 \*                    \*                    \*                    \*                    \*  
 GGG GGC GGA AGC GGC GGA GGG GGA AAC TCC GAA AGG CAT TTC GTG GTC  
 CCC CGG CCT TCG CCG CCT CCC CCT TTG AGG CTT TCC GTC AAG CAC CAG  
 G G G S G G G N S E R H F V V>  
 -- 10 amino acid linker --><----- I-Ad  $\beta$ -1 domain ----->

200                  210                  220                  230                  240  
 \*                    \*                    \*                    \*                    \*  
 CAG TTC AAG GGC GAG TCC TAC TAC ACC AAC GGG ACG CAG CGC ATA CGG  
 GTC AAG TTC CCG CTC ACC ATG ATG TGG TTG CCC TCC GTC GCG TAT GCC  
 Q F K G E C Y Y T N G T Q R I R>  
 ----->

250                  260                  270                  280                  290  
 \*                    \*                    \*                    \*                    \*  
 CTC GTG ACC AGA TAC ATC TAC AAC CGG GAG GAG TAC GTG CGC TAC GAC  
 GAG CAC TGG TCT ATG TAG ATG TTG GCC CTC CTC ATG CAC GCG ATG CTC  
 L V T R Y I Y N R E E Y V R Y D>  
 ----->

300                  310                  320                  330  
 \*                    \*                    \*                    \*  
 ACC GAC GTG GGC GAG TAC CGC GGG GTG ACC GAG CTG GGG CGG CCA GAC  
 TCG CTG CAC CGG CTC ATG GCG CGC CAC TGG CTC GAC CCC CGC GGT CTG  
 S D V G E Y R A V T E L G R P D>  
 ----->

340                  350                  360                  370                  380  
 \*                    \*                    \*                    \*                    \*  
 GGC GAG TAC TGG AAC ACC CAG CGG GAG ATC CTG GAG CGA ACG CGG GCC  
 CGG CTC ATG ACC TTG TCG GTC GGC CTC TAG GAC CTC GCT TGC GCC CGG  
 A E Y W N S Q P E I L E R T R A>  
 ----->

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FIGURE 27 (SHEET 2 OF 4)

390            400            410            420            430

GAG GTG GAC ACG GCG TGC AGA CAC AAC TAC GAG GGG CCG GAG ACC AGC  
CTC CAC CTG TGC CCC ACG TCT GTG TTG ATG CTC CCC CCC CTC TGG TCG  
E V D T A C R H N Y E G P E T S>

---

440            450            460            470            480

ACC TCC CTG CGG CGG CTT GAA CAG CCC AAT GTC GCC ATC TCC CTG TCC  
TGG AGG GAC CCC GCC GAA CTT GTC GGG TTA CAG CGG TAG AGG GAC AGG  
T S L R R L E Q P N V A I S L S>

--- I-Ad  $\beta$ -1 domain --->----- I-Ad  $\beta$ -2 domain -----

---

490            500            510            520            530

AGG ACA GAG GGC CTC AAC CAC AAC ACT CTG GTC TGT TCG GTG AGA  
TCC TGT CTC CGG GAG TTG GTG GTC TTG TGA GAC CAG ACA AGC CAC TGT  
R T E A L N H H N T L V C S V D>

---

540            550            560            570

GAT TTC TAC CCA GCC AAG ATC AAA GTG CCC TGG TTC AGG AAT GGC CAG  
CTA AAG ATG GGT CGG TTC TAG TTT CAC GGG ACC AAG TCC TTA CCG GTC  
D F Y P A K I K V R W F R N G Q>

---

580            590            600            610            620

GAG GAG ACA GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGG GAC  
CTC CTC TGT CAC CCC CAG AGT AGG TGT GTC GAA TAA TCC TTA CCC CTG  
E E T V G V S S T Q L I R N G D>

---

630            640            650            660            670

TGG ACC TTC CAG GTC CTG GTC ATG CTG GAC ATG ACC CCT CAT CAG GGA  
ACC TGG AAC GTC CAG GAC CAG TAC GAC CTC TAC TGG CGA GTA GTC CCT  
W T F Q V L V M L E H T P H Q G>

---

680            690            700            710            720

GAG GTC TAC ACC TGC CAT GTG GAG CAT CCC AGC CTG AAG ACC CCC ATC  
CTC CAG ATG TGC ACC GTA CAC CTC GTA GGG TCG GAC TTC TCG GGG TAG  
E V Y T C H V E H P S L K S P I>

--- I-Ad  $\beta$ -2 domain -----

---

730            740            750            760            770

ACT GTG GAG TGG ACT AGT GGT GGC CCT GGC AGC GGC GGT GGT GGT TCC  
TGA CAC CTC ACC TGA TCA CCA CCA CCG CCA CCG CCA CCA CCA ACC  
T V E W T S G G G G S G G G G G S>

---><----- 24 amino acid linker -----

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FIGURE 27 (SHEET 3 OF 4)

780            790            800            810

GGT GGC GGC GGT TCT GGC GGT TCC TCG ACT GAA GAC GAC ATT  
 CCA CCG CCG CCA AGA CCG CCA CCG CCA AGG AGC TCA CTT CTG CTG TAA  
 G G G G S G G G S S S E D D I>  
 -----><-----

820            830            840            850            860

GAG GGC GAC CAC GTA GGC TTC TAT GGT ACA ACT GTT TAT CAG TCT CCT  
 CTC CGG CTG GTG CAT CCG AAG ATA CCA TGT TGA CAA ATA GTC AGA GGA  
 E A D H V G F Y G T T V Y Q S P>  
 ----- I-Ad  $\alpha$ -1 domain -----

870            880            890            900            910

GGA GAC ATT GGC CAG TAC ACA CAT GAA TTT GAT GGT GAT GAG TTC TTC  
 CCT CTG TAA CCG GTC ATG TGT GTA CTT AAA CTA CCA CTA CTC AAC AAG  
 G D I G Q Y T H E F D G D E L F>  
 -----

920            930            940            950            960

TAT GTG GAC TTG CAT AAG AAG AAA ACT GTC TGG AGG CTT CCT GAG TTT  
 ATA CAC CTG AAC CTA TTC TTC TTT TGA CAG ACC TCC GAA GGA CTC AAA  
 Y V D L D K K K T V W R L P E F>  
 -----

970            980            990            1000            1010

CCC CAA TTG ATA CTC TTT GAG CCC CAA GGT GCA CTG CAA AAC ATA GCT  
 CCG GTT AAC TAT GAG AAA CTC GGG GTT CCA CCT GAC GTT TTG TAT CGA  
 G Q L I L F E P Q G G L Q N I A>  
 -----

1020            1030            1040            1050

GCA GAA AAA CAC AAC TTG GGA ATC TTG ACT AAG AGG TCA AAT TTC ACC  
 CGT CTT TTT GTG TTG AAC CCT TAG AAC TGA TTC TCC AGT TTA AAG TGG  
 A E K H N L G I L T K R S N F T>  
 ----- I-Ad  $\alpha$ -1 domain -----

1060            1070            1080            1090            1100

CCA GCT ACC AAT GAG GGT CCT CAA GCG ACT GTG TTC CCC AAG TCC CCT  
 GGT CGA TGG TTA CTC CGA CGA GTT CGC TGA CAC AAG GGG TTC AGG GGA  
 P A T N E A P Q A T V F P K S P>  
 -----><----- I-Ad  $\alpha$ -2 domain -----

1110            1120            1130            1140            1150

GTG CTG CTG GGT CAG CCC AAC ACC CTT ATC TGC TTT GTG GAC AAC ATC  
 CAC GAC GAC CCA GTC GGG TTG TGG GAA TAG ACG AAA CAC CTG TTG TAG  
 V L L G Q P N T L I C F V D N I>  
 -----

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FIGURE 27 (SHEET 4 OF 4)

1160	1170	1180	1190	1200
<pre> TTC CCA CCT GTG ATC AAC ATC ACA TGG CTC AGA AAT ACC AAG TCA GTC AAG GGT GGA CAC TAG TTG TAG TGT ACC GAG TCT TTA TCG TTC AGT CAG F P P V I N I T W L R H S K S V&gt; -----</pre>				
1210	1220	1230	1240	1250
<pre> ACA GAC GGC GTT TAT GAG ACC AGC TTC CTC GTC AAC CGT GAC CAT TCC TGT CTG CCG CAA ATA CTC TGG TCG AAG GAG CAG TTG GCA CTG GTA AGG T D G V Y E T S F L V N R D H S&gt; -----</pre>				
1260	1270	1280	1290	
<pre> TTC CAC AAG CTG TCT TAT CTC ACC TTC ATC CCT TCT GAT GAT GAC ATT AAG GTG TTC GAC AGA ATA GAG TGG AAG TAG CGA AGA CTA CTA CTG TAA F H K L S Y L T F I P S D D D I&gt; -----</pre>				
1300	1310	1320	1330	1340
<pre> TAT GAC TGC AAG GTG GAG CAC TGG GGC CTG GAG GAG CCG GTT CTG AAA ATA CTG ACG TTC CAC CTC GTG ACC CCG GAC CTC CTC GGC CAA GAC TTT Y D C K V E H W G L E E P V L K&gt; ----- I-Ad a-2 domain -----</pre>				
1350	1360	1370	1380	
<pre> CAC TGG TCC CGG CCT AGT CAC CAT CAC CAT CAC TAG GTG ACC AGG CCC CGA TCA GTG GTA GTG GTA GTG ATC H W S R A S H H H H H H H H *-&gt; -----&gt;&lt;----- 6 X HIS tag -----&gt;</pre>				

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FIGURE 28 (SHEET 1 OF 4)

10            20            30            40            50

CCACC ATG GCT CTG CAG ATC CCC AGC CTC CTC CTC TCA GCT GCT GTG GTG  
GGTGG TAC CGA GAC GTC TAG GGG TCG GAG GAG AGT CGA CGA CAC CAC  
M A L Q I P S L L L S A A V V>  
----- I-Ad  $\beta$  chain leader -----

60            70            80            90

\* \* \* \* \*  
GTC CTG ATG GTG CTG AGC AGC CCA AGG ACC TTA AGT ATC TCT CAG GCT  
CAC GAC TAC CAC GAC TCG TCG GGT TCC TGG AAT TCA TAG AGA GTC CGA  
V L M V L S S P R T L S I S Q A>  
----->

100           110           120           130           140

\* \* \* \* \*  
GTT CAC GCT GCT CAC GCT GAA ATC AAC GAA GCT GGT CGT GCT AGC GGA  
CAA GTG CGA CGA GTG CGA CTT TAG TTG CTT CGA CCA GCA CGA TCG CCT  
V H A A H A E I N E A G R A S G>  
----- OVA 323-339 ----->

150           160           170           180           190

\* \* \* \* \*  
GGG GGC GGA AGC GGC GGA GGG GGA AAC TCC GAA AGG CAT TTC GTG GTC  
CCC CCG CCT TCG CCG CCT CCC CCT TTG AGG CTT TCC GTC AAG CAC CAG  
G G G S G G G G N S E R H F V V>  
-- 10 amino acid linker -->----- I-Ad  $\beta$ -1 domain -----

200           210           220           230           240

\* \* \* \* \*  
CAG TTC AAG GGC GAG TGC TAC TAC ACC AAC GGG AGC CAG CGC ATA CGG  
GTC AAG TTC CCG CTC ACG ATG ATG TGG TTG CCC TGC GTC GCG TAT GCC  
Q F K G E C Y Y T N G T Q R I R>  
-----

250           260           270           280           290

\* \* \* \* \*  
CTC GTG ACC AGA TAC ATC TAC AAC CGG GAG GAG TAC GTG CGC TAC GAC  
GAG CAC TGG TCT ATG TAG ATG TTG CCC CTC CTC ATG CAC GCG ATG CTG  
L V T R Y I Y N R E E Y V R Y D>  
-----

300           310           320           330

\* \* \* \* \*  
AGC GAC GTG GGC GAG TAC CGC GCG GTG ACC GAG CTG GGG CGG CGG CCA GAC  
TGG CTG CAC CGG CTC ATG GCG CGC CAC TGG CTC GAC CCC GCG GGT CTG  
S D V G E Y R A V T E L G R P D>  
-----

340           350           360           370           380

\* \* \* \* \*  
GCC GAG TAC TGG AAC ACC CAG CGG GAG ATC CTG GAG CGA ACG CGG CGC  
CGG CTC ATG ACC TTC TCG GTC GGC CTC TAG GAC CTC GCT TGC GCC CGG  
A E Y W N S Q P E I L E R T R A>  
-----

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FIGURE 28 (SHEET 2 OF 4)

390            400            410            420            430

GAG GTG GAC ACG GCG TGC AGA CAC AAC TAC GAG GGG CCG GAG ACC AGC  
CTC CAC CTG TGC CGC ACG TCT GTG TTG ATG CTC CCC CGC CTC TGG TCG  
E V D T A C R H N Y E G P E T S>

---

440            450            460            470            480

ACC TCC CTG CGG CGG CTT GAA CAG CCC AAT GTC CCC ATC TCC CTG TCC  
TGG AGG GAC GCC GCC GAA CTT GTC GGG TTA CAG CGG TAG AGG GAC AGG  
T S L R R L E Q P N V A I S L S>

--- I-Ad  $\beta$ -1 domain --->--- I-Ad  $\beta$ -2 domain -----

---

490            500            510            520            530

ACG ACA GAG GCC CTC AAC CAC CAC AAC ACT CTC GTC TGT TCG TCG ACA  
TCC TGT CTC CGG GAG TTG GTG GTG TTG TGA GAC CAC ACA AGC CAC TGT  
R T E A L N H H N T L V C S V T>

---

540            550            560            570

GAT TTC TAC CCA GCC AAG ATC AAA GTG CGC TGG TTC AGG AAT GGC CAG  
CTA AAG ATG GGT CGG TTC TAG TTT CAC CGG ACC AAG TCC TTA CCG GTC  
D F Y P A K I K V R W F R N G Q>

---

580            590            600            610            620

GAG GAG ACA GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGG GAC  
CTC CTC TGT CAC CCC CAG AGT AGG TGT GTC GAA TAA TCC TTA CCC CTC  
E E T V G V S S T Q L I R N G D>

---

630            640            650            660            670

TGG ACC TTC CAG GTC CTG GTC ATG CTG GAG ATG ACC CCT CAT CAG CGA  
ACC TGG AAG GTC CAG GAC CAG TAC GAC CTC TAC TGG CGA GTA GTC CCT  
W T F Q V L V M L E M T P H Q G>

---

680            690            700            710            720

GAG GTC TAC ACC TGC CAT GTG GAG CAT CCC ACC CTG AAG ACC CCC ATC  
CTC CAC ATG TGG ACC GTA CAC CTC GTC GCA CGG TCG GAC TTC TCG GGG TAC  
E V Y T C H V E H P S L K S P I>

--- I-Ad  $\beta$ -2 domain -----

---

730            740            750            760            770

ACT GTG GAG TGG ACT ACT GGT GGC GGT GGC AGC GGC GGT GGT GGT TCC  
TCA CAC CTC ACC TGA TCA CCA CGG CCA CGG TCG CGG CCA CCA CCA AGG  
T V E W T S G G G S G G G G G S>

-----><----- 24 amino acid linker -----

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## FIGURE 28 (SHEET 3 OF 4)

780            790            800            810  
 GGT GGC GGC GGT TCT GGC GGT TCC TCG ACT GAA GAC GAC ATT  
 CGA CCG CCG CCA AGA CCG CCA AGG AGC TCA CTT CTG CTG TAA  
 G G G G S G G G S S S E D D I>  
 -----><-----  
 820            830            840            850            860  
 GAG GCC GAC CAC GTA GGC TTC TAT GGT ACA ACT GTT TAT CAG TCT CCT  
 CTC CCG CTG GTG CAT CCG AGG ATA CCA TGT TGA CAA ATA GTC AGA GGA  
 E A D H V G F Y G T T V Y Q S P>  
 ----- I-Ad  $\alpha$ -1 domain -----  
 870            880            890            900            910  
 GGA GAC ATT GGC CAG TAC ACA CAT GAA TTT GAT GGT CAT GAG TTG TTC  
 CCT CTG TAA CCG GTC ATG TGT GTA CTT AAA CTA CCA CTA CTC AAC AAG  
 G D I G Q Y T H E F D G D E L F>  
 -----  
 920            930            940            950            960  
 TAT GTG GAC TTG GAT AAG AAG AAA ACT GTC TCG AGG CTT CCT GAG TTT  
 ATA CAC CTG AAC CTA TTC TTC TTT TGA CAG ACC TCC GAA GGA CTC AAA  
 Y V D L D K K T V W R L P E F>  
 -----  
 970            980            990            1000            1010  
 GGC CAA TTG ATA CTC TTT GAG CCC CAA GGT GGA CTG CAA AAC ATA GCT  
 CCG GTT AAC TAT GAG AAA CTC GGG GTT CCA CCT GAC GTT TTG TAT CGA  
 G Q L I L F E P Q G G E Q N I A>  
 -----  
 1020            1030            1040            1050  
 GCA GAA AAA CAC AAC TTG GGA ATC TTG ACT AAG AGG TCA ATC TTC ACC  
 CGT CTT TTT GTG TTG AAC CCT TAG AAC TGA TTC TCC ACT TTA AAG TGG  
 A E K H N L G I L T K R S N F T>  
 ----- I-Ad  $\alpha$ -1 domain -----  
 1060            1070            1080            1090            1100  
 CCA GCT ACC AAT GAG GCT CCT CAA GCG ACT GTC TTC CCC AAG TCC CCT  
 GGT CGA TGG TTA CTC CGA GGA GTT CGC TGA CAC AAG GGG TTC AGG GGA  
 P A T N E A P Q A T V F P K S P>  
 -----><----- I-Ad  $\alpha$ -2 domain -----  
 1110            1120            1130            1140            1150  
 GTG CTG CTG GGT CAG CCC AAC ACC CTT ATC TGC TTT GTG GAC AAC ATC  
 CAC GAC GAC CCA GTC GGG TTG TGG GAA TAG ACG AAA CAC CTG TTG TAG  
 V L L G Q P N T L I C F V D N I>

## FIGURE 28 (SHEET 4 OF 4)

1160            1170            1180            1190            1200  
 TTC CCT CCT GTG ATC AAC ATC ACA TGG CTC AGA AAT AGC AAG TCA GTC  
 AAC GGT GGA CAC TAG TTC TAG TGT ACC GAG TCT TTA TCG TTC AGT CAG  
 F P P V I N I T W L R N S K S V>  
 -----  
 1210            1220            1230            1240            1250  
 ACA GAC GGC GTT TAT GAG ACC AGC TTC CTC GTC AAC CGT GAC CAT TCC  
 TGT CTG CCG CAA ATA CTC TGG TCG AAG GAG CAG TTG GCA CTG GTA AGG  
 T D G V Y E T S F L V N R D H S>  
 -----  
 1260            1270            1280            1290  
 TTC CAC AAG CTG TCT TAT CTC ACC TTC ATC CCT TCT GAT GAT GAC ATT  
 AAG GTG TTC GAC AGA ATA GAG TGG AAG TAG GGA AGA CTA CTA CTG TAA  
 F H K L S Y L T F I P S D D D I>  
 -----  
 1300            1310            1320            1330            1340  
 TAT GAC TGC AAG GTG GAG CAC TGG CCC CTC GAG GAG CCG GTT CTC AAA  
 ATA CTG ACG TTC CAC CTC GTG ACC CCG GAC CTC CTC GGC CAA GAC TTT  
 Y D C K V E H W G L E E P V L K>  
 ----- I-Ad  $\alpha$ -2 domain -----  
 1350            1360            1370            1380            1390  
 CAC TGG CAA CCT GAG ATT CCA GGC CCC ATG TCA GAG CTG ACA GAA ACT  
 GTG ACC CTT GGA CTC TAA GGT CCG GGG TAC AGT CTC GAC TGT CTT TGA  
 H N E P E I P A P M S E L T E T>  
 -----><----- I-Ad  $\alpha$ -TM domain -----  
 1400            1410            1420            1430            1440  
 GTG GTG TGT GCC CTG GGG TTG TCT GTG GGC CTT GTG GGC ATC GTG GTG  
 CAC CAC ACA CGG GAC CCC AAC AGA CAC CCG GAA CAC CGG TAG CAC CAC  
 V V C A L G L S V G L V G I V V>  
 -----  
 1450            1460            1470            1480            1490  
 GGC ACC ATC TTC ATC ATT CAA GGC CTG CGA TCA GGT GGC ACC TCC AGA  
 CGG TGG TAG AAG TAG TAA GTT CCG GAC GCT AGT CCA CGG TGG AGG TCT  
 G T I F I I Q G L R S G G T S R>  
 -----  
 1500  
 CAC CCA CGG CCT TTA TGA  
 GTG GGT CCC GGA AAT ACT  
 H P G P L >  
 - I-Ad  $\alpha$ -TM domain ->

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FIGURE 29 (SHEET 1 OF 4)

10                    20                    30                    40                    50

CCACC ATG GCT CTG CAG ATC CCC AGC CTC CTC CTC TCA GCT GCT GTG GTG  
GGTGG TAC CGA GAC GTC TAG GGG TCG GAG GAG GAG ACT CGA CGA CAC CAC  
M A L Q I P S L L L S A A V V>  
----- I-Ad  $\beta$  chain leader -----

60                    70                    80                    90

GTG CTG ATG GTG CTG AGC AGC CCA AGG ACC TTA AGT ATC TCT CAG GCT  
CAC GAC TAC CAC GAC TCG TCG GGT TCC TCG AAT TCA TAG AGA GTC CCA  
V L M V L S S P R T L S I S Q A>  
----->

100                    110                    120                    130                    140

GTT CAC GCT CAC GCT GAA ATC AAC GAA GCT GGT CGT GCT AGC GGA  
CAA GTG CGA CGA GTG CGA CTT TAG TTG CTT CGA CCA GCA CGA TCG CCT  
V H A A H A E I N E A G R A S G>  
----- OVA 323-339 -----><-----

150                    160                    170                    180                    190

GGG GGC GGA AGC GGC GGA GGG GGA AAC TCC GAA AGG CAT TTC GTG GTC  
CCC CCG CCT TCG CCG CCT CCC CCT TTG AGG CTT TCC GTC AAG CAC CAG  
G G G S G G G G N S E R H F V V>  
-- 10 amino acid linker --><----- I-Ad  $\beta$ -1 domain -----

200                    210                    220                    230                    240

CAG TTC AAG GGC GAG TGC TAC TAC ACC AAC GGG AGC CAG CGC ATA CGG  
GTC AAG TTC CCG CTC ACG ATG ATG TGG TTG CCC TGC GTC GCG TAT GCC  
Q F K G E C Y Y T N G T Q R I R>  
-----

250                    260                    270                    280                    290

CTC GTG ACC AGA TAC ATC TAC AAC CGG GAG GAG TAC GTG CGC TAC GAC  
GAG CAC TGG TCT ATG TAG ATG TTG GCC CTC CTC ATG CAC GCG ATG CTG  
L V T R Y I Y N R E E Y V R Y D>  
-----

300                    310                    320                    330

AGC GAC GTG GGC GAG TAC CGC GCG GTG ACC GAG CTG GGG CGG CCA GAC  
TCG CTG CAC CGC CTC ATG GCG CGC CAC TGG CTC GAC CCC CGC GGT CTC  
S D V G E Y R A V T E L G R P D>  
-----

340                    350                    360                    370                    380

GCC GAG TAC TGG AAC AGC CAG CGG GAG ATC CTG GAG CGA ACG CGG CGC  
CGG CTC ATG ACC TTG TCG GTC CGC CTC TAG GAC CTC GCT TGC CGC CGG  
A E Y W N S Q P E I L E R T R A>  
-----

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FIGURE 29 (SHEET 2 OF 4)

390            400            410            420            430  
 \*            \*            \*            \*            \*  
 GAG GTG GAC ACC GCG TGC AGA CAC AAC TAC GAG GGG CCG GAC ACC AGC  
 CTC CAC CTG TGC CGC ACC TCT GTG TTG ATG CTC CCC GGC CTC TGG TCG  
 E V D T A C R H N Y E G P E T S>  
 -----  
 440            450            460            470            480  
 \*            \*            \*            \*            \*  
 ACC TCC CTG CGG CGG CTT GAA CAG CCC AAT GTC GCC ATC TCC CTG TCC  
 TGG AGG GAC GCC CCC GAA CTT GTC GGG TTA CAG CGG TAG AGG GAC AGG  
 T S L R R L E Q P N V A I S L S>  
 --- I-Ad  $\beta$ -1 domain ->----- I-Ad  $\beta$ -2 domain -----  
 490            500            510            520            530  
 \*            \*            \*            \*            \*  
 AGG ACA GAG GCG CTC AAC CAC CAC AAC ACT CTG GTC TGT TCG GTG ACA  
 TCC TGT CTC CGG GAG TTG GTG GTG TTG TGA GAC CAG ACA ACC CAC TGT  
 R T E A L N H H N T L V C S V T>  
 -----  
 540            550            560            570  
 \*            \*            \*            \*  
 GAT TTC TAC CCA CCC AAG ATC AAA GTG CGC TGG TTC AGG AAT GGC CAG  
 CTA AAG ATG GGT CGG TTC TAG TTT CAC CGG ACC AAG TCC TTA CCG GTC  
 D F Y P A K I K V R W F R N G Q>  
 -----  
 580            590            600            610            620  
 \*            \*            \*            \*            \*  
 GAG GAG ACA GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGC GAC  
 CTC CTC TGT CAC CCC CAG AGT AGG TGT GTC GAA TAA TCC TTA CCC CTC  
 E E T V G V S S T Q L I R N G D>  
 -----  
 630            640            650            660            670  
 \*            \*            \*            \*            \*  
 TCG ACC TTC CAG GTC CTG GTC ATG CTG GAG ATG ACC CCT CAT CAG GGA  
 ACC TCG AAG GTC CAG GAC CAG TAC GAC CTC TAC TGG GGA GTA GTC CCT  
 W T F Q V L V M L E M T P H Q G>  
 -----  
 680            690            700            710            720  
 \*            \*            \*            \*            \*  
 GAG GTC TAC ACC TGC CAT GTG GAG CAT CCC AGC CTG AAG AGC CCC ATC  
 CTC CAG ATG TGG AGC GTA CAC CTC GTA GGG TCG GAC TTC TCG GGG TAC  
 E V Y T C H V E H P S L K S P I>  
 ----- I-Ad  $\beta$ -2 domain -----  
 730            740            750            760            770  
 \*            \*            \*            \*            \*  
 ACT GTG GAG TGG ACT ACT GGT GGC GGT GGC AGC GGC GGT GGT GGT TCC  
 TGA CAC CTC ACC TGA TCA CCA CGG CCA CGG TCG CGG CCA CCA CCA AGG  
 T V E W T S G G G G S G G G G G S>  
 -----><----- 24 amino acid linker -----

## FIGURE 29 (SHEET 3 OF 4)

780            790            800            810  
 CCT GCC GGC GGT TCT GGC GGT GGC GGT TCC TCG ACT GAA GAC GAC ATT  
 CCA CGG CGG CCA AGA CCG CCA CGG CCA AGG AGC TCA CTT CTG CTG TAA  
 G G G G S G G G G S S S S E D D I>  
 ----->

820            830            840            850            860  
 GAG GCC GAC CAC GTA GGC TTC TAT GGT ACA ACT GTT TAT CAG TCT CCT  
 CTC CGG CTG GTG CAT CGG AAG ATA CCA TGT TGA CAA ATA GTC AGA GGA  
 E A D H V G F Y G T T V Y Q S P>  
 ----- I-Ad  $\alpha$ -1 domain -----

870            880            890            900            910  
 GGA GAC ATT GGC CAG TAC ACA CAT GAA TTT GAT GGT GAT GAG TTG TTC  
 CCT CTG TAA CCG GTC ATG TGT GTA CTT AAA CTA CCA CTA CTC AAC AAG  
 G D I G Q Y T H E F D G D E L F>  
 ----->

920            930            940            950            960  
 TAT GTG GAC TTG GAT AAG AAG AAA ACT GTC TGG AGG CTT CCT GAG TTT  
 ATA CAC CTG AAC CTA TTC TTC TTT TGA CAG ACC TCC GAA GGA CTC AAA  
 Y V D L D K K T V W R L P E F>  
 ----->

970            980            990            1000            1010  
 GGC CAA TTG ATA CTC TTT CAG CCC CAA GGT GGA CTG CAA AAC ATA GCT  
 CGG GTT AAG TAT GAG AAA CTC CGG GTT CCA CCT GAC GTT TTG TAT CCA  
 G Q L I L F E P Q G G L Q N I A>  
 ----->

1020            1030            1040            1050  
 GCA GAA AAA CAC AAC TTG GGA ATC TTG ACT AAG AGG TCA AAT TTC ACC  
 CCT CTT TTT GTG TTG AAC CCT TAG AAC TGA TTC TCC ACT TTA AAG TGG  
 A E K H N L G I L T K R S N F T>  
 ----- I-Ad  $\alpha$ -1 domain -----

1060            1070            1080            1090            1100  
 CCA GGT ACC AAT GAG GCT CCT CAA GCG ACT GTG TTC CCC AAG TCC CCT  
 GGT CGA TGG TTA CTC CGA CGA GTT CGC TGA CAC AAG GGG TTC AGG CGA  
 P A T N E A P Q A T V F P K S P>  
 -----> I-Ad  $\alpha$ -2 domain -----

1110            1120            1130            1140            1150  
 GTG CTG CTG GGT CAG CCC AAC ACC CTT ATC TGC TTT GTG GAC AAC ATC  
 CAC GAC GAC CCA GTC CGG TTG TGG GAA TAG AGC AAA CAC CTG TTG TAG  
 V L L G Q P N T L I C F V D N I>  
 ----->

## FIGURE 29 (SHEET 4 OF 4)

1160	1170	1180	1190	1200
TTC CCA CCT GTG ATC AAC ATC ACA TGG CTC AGA AAT AGC AAG TCA GTC AAG GGT GGA CAC TAG TTG TAG TGT ACC GAG TCT TTA TCG TTC AGT CAG F P P V I N I T W L R N S K S V>				
<hr/>				
1210	1220	1230	1240	1250
ACA GAC GGC GTT TAT GAG ACC AGC TTC CTC GTC AAC CGT GAC CAT TCC TGT CTG CCG CAA ATA CTC TGG TCG AAG GAG CAG TTG GCA CTG GTA AGG T D G V Y E T S F L V N R D H S>				
<hr/>				
1260	1270	1280	1290	
TTC CAC AAG CTG TCT TAT CTC ACC TTC ATC CCT TCT GAT GAT GAC ATT AAG GTG TTC GAC AGA ATA GAG TGG AAG TAG GGA AGA CTA CTA CTG TAA F H K L S Y L T F I P S D D D I>				
<hr/>				
1300	1310	1320	1330	1340
TAT GAC TGC AAG GTG GAG CAC TGG GGC CTG GAG GAG CCG GTT CTG AAA ATA CTG ACG TTC CAC CTC GTG ACC CCG GAC CTC CTC GGC CAA GAC TTT Y D C K V E H W G L E E P V L K>				
----- I-Ad $\alpha$ -2 domain -----				
1350	1360	1370	1380	
CAC TGG GAG GAA GAA GAG TAC CCG ATG GAA TGA GTG ACC CTC CTT CTT CTC ATG TAC GGC TAC CTT ACT H W E E E E Y M P M E *>				
----- EE tag ----->				